The British Veterinary Journal

SEPTEMBER, 1958

CONTENTS

(The Whole of the Literary matter in The British Veterinary Journal is Copyright)

PAGE

EDITORIAL

The Isle of Man 317

The Annual B.V.A. Congress, 1958, at Douglas, I. of M. .. 319

GENERAL ARTICLES—

The Effect of Feeding Two Levels of Concentrates on the Incidence of Ketosis in Dairy Cows

By P. S. Blackburn, M. E. Castle and N. H. Strachan .. 323

The Effect of Antibiotic Supplements on the Faecal Streptococci (Lancefield Group D) of Poultry

By Ella M. Barnes 333

Hepatic Veno-Occlusive Disease and Megalocytosis in *Senecio* Poisoning of Horses

By Kenneth R. Hill, B.Sc., M.D., M.R.C.P., and H. M. Martin, V.M.D., A.M., Ph.D. 345

Studies on Intervertebral Disc Protrusion in the Dog

By L. C. Vaughan 350

Some Characteristics of Type A Strains of Pasteurella Multocida

By G. R. Carter, D.V.M., M.S., D.V.Sc. 356

Tuberculosis in a Wallaby Caused by the Avian Type Tubercle Bacillus

By I. W. Lesslie, B.V.Sc., M.R.C.V.S. 358

Rupture of the Gastrocnemius Tendon in Fowls

By R. B. A. Carnaghan and B. S. Hanson .. 360

REVIEWS 366

NEWS 368

NOTICES 368

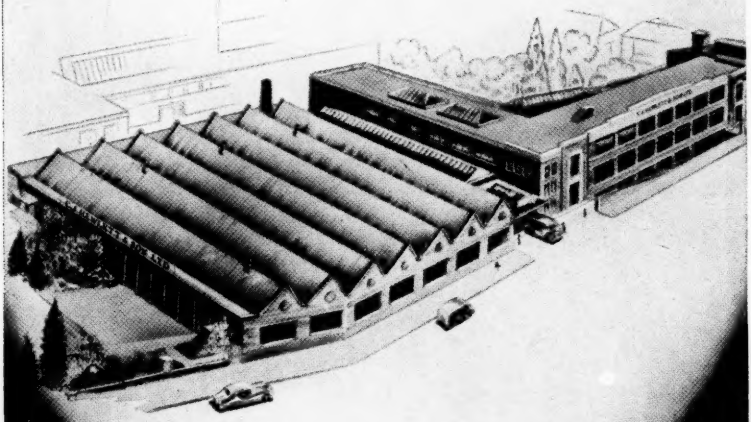
NOTICE TO CONTRIBUTORS

Please turn to the inside back cover of this **Journal** *for particulars regarding submission of articles to the Editors.*

XUM

ESTABLISHED 1832

DRUGS AND FINE CHEMICALS
VETERINARY SPECIALITIES
AND INSTRUMENTS



C. J. HEWLETT & SON LTD.
MANUFACTURING CHEMISTS
KING GEORGE'S AVENUE, WATFORD, HERTS.
Telegrams: Pepsine, Watford Telephone: Watford 34401

STAND NO. 11

THE BRITISH VETERINARY JOURNAL

EDITORS :

J. McCUNN, *F.R.C.S., L.R.C.P., M.R.C.V.S.*

R. LOVELL, *D.Sc., Ph.D., M.R.C.V.S., D.V.S.M.*

SIR THOMAS DALLING, *M.A., D.Sc., F.R.C.V.S., F.R.S.E.*

Vol. 114 **September, 1958** **No. 9**

The whole of the matter appearing in "The British Veterinary Journal" is Copyright and may not be reproduced in whole or in part without permission.

EDITORIAL

THE ISLE OF MAN

A WELCOME oasis set in a turbulent sea, its people modern and progressive yet steeped in the customs and traditions of the past. Archaeologists can trace its history to the mesolithic period 6000 B.C. It was inhabited in early times by people of Scoto-Irish origin and was a great centre of Druidism. It was the centre of contention and trouble arising from the rival claims to possession of its greater Celtic neighbours. It was the scene of a war in 503 after which it came under the domain of Maelgwyn, King of North Wales. It continued under the sovereignty of his son but after a great battle in 581 it passed under the domain of Aodan McGrabban, King of the Scots. It remained under the Scots until Edwin, King of Northumbria, took it from them in 625. The Northumbrian ruled until his death in 633 when it reverted to the Scots. The Scots at that time were troubled with their own problems of disputed succession and the island was for many years the area of trouble and strife until in 825 it reverted to the domain of North Wales.

Wales had troubles also and when in 877 their Kingdom was divided into three parts, Man was assigned to Prince Anaraud and made a separate Kingdom. It was ravaged by Norsemen constantly and to help keep them at bay Anaraud assigned himself to the protection of Alfred the Great of England. Anaraud was the last of the Welsh lineage to reign in Man.

In 888 Harold Haarfager, King of Norway, who had already subjugated the Orkneys and Hebrides, invaded the island and drove Anaraud from his throne. Harold established Vice-regal rule, naming Tarl Ketit Bjornson as Viceroy. Tarl was ambitious and usurped the powers of King or Prince and in 890 the island became an independent Kingdom again. And so it remained until the islanders rebelled against his grandson and expelled him but they could not agree amongst themselves and for some time there was no settled central government. The Danes were the next to cast envious eyes upon the land and one Orrey, a marauder who had over-run the Orkneys and Hebrides, arrived off the coast with a great fleet and, no doubt in the hope that their troubles might end, the islanders accepted him as King. This was early in the

XUM

10th century. Olave, Fingal and Godred followed Orrey, until in 973 Macon, a son of the King of Dublin and an admiral of Edgar of England, came with a powerful British fleet and took possession of the island. And so the islanders' sorry tale goes on. They were the catspaws for many generations of the Danes, Norwegians, Icelanders, Scots, English, Irish and Welsh. In time the dispute as to the possession was narrowed so that the contending parties were the English and the Scots. Sometimes one, sometimes the other held sway and the independent and courageous islanders rebelled against both.

In 1333 a female descendant of the last King of Man made claim to the sovereignty and appealed to Edward III of England for protection. He recognised her right but, no doubt with the idea of claiming Man as part of his own Kingdom, he arranged that she should marry and, as her spouse, he chose one of his own men, Sir William de Montacute, who was created Earl of Salisbury at a later date. The Scots unseated Montacute and regained possession but the islanders were favourable to Sir William for by his marriage to their lady they accepted him as a rightful Prince. Montacute regained possession but in this effort his fortunes were so depleated that he had to mortgage the island to the Bishop of Durham for a period of seven years. The island has remained with England since that time. The second Earl of Salisbury sold it to the Earl of Wiltshire. When Lord Wiltshire was attained it was given to Henry Percy, Earl of Northumberland. When in turn Northumberland was attained it passed to Sir John Stanley in 1406. Sir John's descendants became Earls of Derby. When the seventh Earl was executed in 1651 it was lost again and seized by the Parliamentary forces. At the Restoration the family regained possession and held it until the death, without issue, of the tenth Earl in 1735. It then passed to James Murray, second Duke of Atholl, by virtue of him being a descendant of a daughter of the second Earl of Derby. Subsequently, in 1825, the British Government acquired the Atholl interests in the island by the payment of a sum of money (said to have been £416,114).

This brief and incomplete review of the known history of the island brings one almost to modern times, when Man became a Crown Colony. The island is to some extent independent of the central government and acts of the Imperial Government do not extend to the island unless they be specifically mentioned. Authority is vested in a Lieutenant Governor, a Council, and a body called the House of Keys which is made up of 24 elected members. The island has its own particular courts of justice and proclamations are made in Manx and English.

The chief industries are agriculture, fishing and catering for visitors. The island has been a popular holiday resort for many years and visitors can be sure of a great welcome. The scenery of the countryside is picturesque and Man is famed for its hills and glens. Highland Cattle and Manx sheep are to be seen on the hills, from which one can look over the sea to the lands of the English, Scots, Welsh and Irish who strove for possession in the turbulent past.

There are mediaeval castles and lovely fishing villages to visit where much of the way of life is as of old and the Manx language can be heard still. For those who prefer more modern forms of relaxation the capital town, Douglas, can provide all that can possibly be desired. Entertainment is not costly and liquid "cheer" is cheaper than on the mainland and the hours of licence extend from 10 a.m. to 11 p.m. In these hard times a holiday in Man offers many advantages. Visitors to the Congress can be assured that, in the intervals between their bouts of academic fare or if they become satiated with the latter, they will be able to find much of interest and delight in this pleasant and happy island.

XUM

BRITISH VETERINARY ASSOCIATION CONGRESS: DOUGLAS, I.o.M.

SEPTEMBER 1958

PROGRAMME

Sunday, September 21st

Time	Congress Social Events, etc.	Scientific Sessions	Tours, Excursions, etc.
	NOTE.—After the arrival of the Saturday boat from Liverpool, there will be no incoming boat on Sunday.		
p.m. 2.15	(Congress Office open till 6.0 p.m. for registrations —Palace Buildings)		Tour of T.T. Course *or* Angling Excursion
6.30	Choral Evensong, St. Thomas's Church		
8.0	**Council Meeting** (Villa Marina)		
8.30	Lancashire Division **"At Home"** (Villa Marina)		
10.0 to midnight	Film Show in aid of Benevolent Funds (Villa Marina)		

Monday, September 22nd

a.m. 10.0	**Official Opening of Congress and Civic Welcome** from the Deputy Governor, Isle of Man, and the Mayor of Douglas Followed by:—		
11.0	**Opening of Trade Exhibition**		
12.0	**Annual General Meeting and Presidential Address**		

Monday, September 22nd—contd.

Time	Congress Social Events, etc.	Scientific Sessions		Tours, Excursions, etc.
p.m. 2.30		Metabolic diseases of the ruminant 1. In relation to husbandry *Author*: Mr. L. E. Hughes 2. Vitamin D in milk Fever *Author*: Mr. C. Weighton 3. Acetonaemia *Author*: Dr. J. R. Holmes *Chairman*: Mr. H. F. Hebeler *Openers*: Mr. D. T. Michael, Mr. G. B. Stubbs	Skin conditions met with in small-animal practice *Author*: Mr. J. A. Dall *Chairman*: Mr. H. Hewetson *Opener*: Mr. E. F. Lewis	Ladies' Excursion to Ramsey via Snaefell. Visit to "Aristoc" stocking factory. Sulby Glen to Tholt-y-Will. Mooragh Park for Tea
5.0		Films "The Veterinary Surgeon in Civil Defence"		
8.0	**Mayor's Reception and Dance** (Villa Marina)			
Tuesday, September 23rd				
a.m. 9.30		Salmonellosis in animals *Author*: Dr. A. Buxton *Chairman*: Prof. E. White *Opener*: Mr. H. I. Field	Nervous diseases in the dog *Author*: Mr. E. M. Pittaway *Chairman*: Mr. S. Jennings *Opener*: Mr. W. T. Harrow	
10.30				Ladies' Annual General Meeting, followed by coffee
12 noon		**Plenary Session** **Livestock Improvement in Britain** *Author*: Prof. M. M. Cooper *Chairman*: Mr. E. R. Callender		

XUM

Tuesday, September 23rd—contd.

Tuesday, September 23rd—comtd.

Time	Congress Social Events, etc.	Scientific Sessions		Tours, Excursions, etc.
p.m. 2.30				Ladies' Excursion to Peel City and Castle, via St. John's and Tynwald Hill, Fish Hatchery, Michael; return via Glen Helen. Grainger's Café, Peel, for tea
		The incidence of respiratory diseases of poultry *Author*: Dr. H. P. Chu *Chairman*: Dr. G. Davies *Opener*: Dr. F. Jordan	Mycotic Dermatitis of sheep in Britain *Authors*: Mr. K. Austwick & E. T. Davies *Chairman*: Mr. W. Rolands *Opener*: Mr. G. B. S. Heath	
5.0		Films: " Veterinary Anaesthesia "		
8.0 to 1 a.m.	**Ladies' Guild Dance** (Villa Marina)			
Wednesday, September 24th				
a.m. 9.30		A.I. of cattle: Its development and future *Author*: Mr. G. F. Smith *Chairman*: Mr. J. Moss *Openers*: Mr. J. Bruford Mr. D. H. L. Madden	The cystic hyperplasia hyometra complex in the bitch *Author*: Mr. C. Dow *Chairman*: Mr. A. Shuttleworth *Opener*: Mr. J. W. Heney	
10.30				Ladies' Excursion — Port Erin Fish Hatchery, Port St. Mary to Cregneish and the Sound.
12 noon		**Plenary Session** **Placental Transference of Antibodies** *Author*: Prof. F. W. R. Brambell *Chairman*: Mr. A. Thomson (Beckenham)		
p.m. 2.15		Films of general interest	**Demonstrations** at I.o.M. Board of Agriculture and Fisheries Experimental Farm 1. Artificial insemination (Mr. G. F. Smith) 2. Meat inspection techniques (Mr. J. M. Olivant)··· 3. Mycotic dermatitis (Mr. P. K. C. Austwick)	Visit to the I.o.M. Board of Agriculture and Fisheries Experimental Farm for Exhibition of Manx tweeds, sheep, cats, and kipper-making. Afternoon tea provided by the Board at 4.30 p.m.
7.45 for 8.0	**Banquet** (Villa Marina)			

Thursday, September 25th

Time	Congress Social Events, etc.	Scientific Sessions		Tours, Excursions, etc.
a.m. 9.30		Diseases of the external auditory meatus in the dog and cat *Author*: Miss J. O. Joshua *Chairman*: Mr. G. Stubbs *Opener*: Mr. T. Denholm	The role of the veterinarian in public health *Author*: Mr. C. G. Allen *Chairman*: Mr. W. Tweed *Openers:* Dr. J. M. Ross Mr. H. E. Bywater	
10.30				Ladies' Excursion to Manx Museum escorted by R. B. Moore, Esq., O.B.E.
12 noon		**Plenary Session** **The Global Picture of Animal Disease** *Author*: Sir Thomas Dalling *Chairman*: Mr. J. McC. Ingram		
p.m. 2.15				Golf Competition (Pulrose Links). Organiser: Mr. R. H. Hawkins.
2.30		Disorders of the lactating sow and new-born pig *Author*: Mr. N. H. Brooksbank *Chairman*: Mr. D. Oliver *Opener*: Dr. D. Luke	Fore-limb lameness in the horse *Author*: Dr. A. C. Fraser *Chairman*: Mr. T. S. Anderson *Opener*: Mr. J. S. M. Cosgrove	Ladies' Excursion via Manx Electric Railway Coastal run to Ramsey (1¼ hours each way) *or* (Weather permitting) Optional Ladies' tour to summit of Snaefell via Mountain Railway
5.0		Film		
8.0	**Reception by Chairman and Members of I.o.M. Board of Agriculture and Fisheries** (Gaiety Theatre)			

Friday, September 26th

a.m. 9.30		The responsibilities of the veterinary surgeon *Author*: Mr. C. C. Stevens *Chairman*: Mr. H. Sumner *Opener*: Dr. J. K. Walley		
10.30				Ladies' Excursion to Castle Rushen
12 noon		Congress Photograph		
p.m. 2.30		Dystokia in Cattle *Author*: Dr. L. G. Anderson *Chairman*: Prof. J. G. Wright *Opener*: Mr. H. Holroyd		Ladies' Excursion: Mystery Tour. Afternoon tea provided Ellerslie Farms, Ltd.
5.0	**Adjourned Annual General Meeting and Installation of New President**			
8.0 to 1 a.m.	**President's Reception**			

THE EFFECT OF FEEDING TWO LEVELS OF CONCENTRATES ON THE INCIDENCE OF KETOSIS IN DAIRY COWS

By

P. S. BLACKBURN, M. E. CASTLE and N. H. STRACHAN

The Hannah Dairy Research Institute, Kirkhill, Ayr.

Ketosis, although rarely fatal, causes high losses to the dairy farmer by lowering milk production and by producing off-flavoured milk and a general loss of condition and body weight in the cow. In the herd in which the following experiment was conducted, ketosis had been a major problem for at least the two previous winters. The herd was fed mainly on home-produced grassland conservation products and a series of trials have now been started to investigate this problem. This paper reports the first two trials in which the effects of changes in the weight of concentrates fed before calving, and the addition of fresh grass to the diet on the incidence and severity of ketosis were investigated.

Experimental

Cows

Twelve Ayrshire cows which were due to calve in the period late February to early April were used. In early January the cows were paired so that the members of each pair were as similar as possible in liveweight, age, length of previous dry period, date of calving and management during dry period. Details are given in Table 1. The animals in each pair were allocated at random to one or other of the two experimental treatments.

Feeding

From January 7th all the twelve cows were offered 28 lb. of grass silage and 8 lb. of dried grass per day. From January 21st to February 18th the amount of dried grass was increased to 12 lb. per day but thereafter it remained constant at 10 lb. per day until spring grazing began on March 26th. For 1 week after that date 4 lb. dried grass were fed per day. The silage continued to be fed until April 15th.

Oat straw was offered to the cows for 9 days at the beginning of the experiment, but since such small amounts were eaten, the offering of oat straw was stopped.

Before calving the cows were offered one of the two following 'steaming-up' rations:—

	Weight of concentrates (lb./cow/day)						
Weeks before calving ...	6	5	4	3	2	1	*Total in 6 weeks (lb.)*
Treatment High level ...	2	4	6	6	8	10	252
Low level ...	2	2	2	2	4	6	126

The composition of the concentrate mixture and of the grass products is given in Table 2. The starch equivalent and digestible crude protein content of the concentrate mixture was calculated from the feeding stuff tables of Woodman (1954).

Adjourned Annual General Meeting and Installation of New President
President's Reception
8.0 to 1 a.m.

TABLE 1

Details of the cows in the two groups, one of which received a high level of concentrates and the other a low level.

Cow number	Liveweight (lb.) 5 weeks before calving	Age Jan.1957 (years)	Length of dry period (weeks)	Date of calving 1957
High level of concentrates				
1	1400	7½	20 *	23 Feb.
2	1272	7½	21 *	1 Mar.
3	1424	9½	12	6 Mar.
4	1136	4½	14	16 Mar.
5	1092	7½	25	6 Apr.
6	1052	5½	13	20 Mar.
Mean	1229	6½	18	
Low level of concentrates				
7	1465	6½	18 *	15 Feb.
8	1337	6½	26 *	26 Feb.
9	1332	9½	12	12 Mar.
10	1205	7	11	12 Mar.
11	1092	9½	12	23 Mar.
12	1100	4½	13	7 Apr.
Mean	1255	7½	15	

* Out at grass with no other supplementary food until 17 December, 1956.

TABLE 2

The composition and calculated feeding values of the concentrate mixture and the roughages.

Concentrate mixture			
	% by weight	Composition as fed	
Barley (ground)	27		
Oats (bruised)	18	Dry matter (%)	88.0
Flaked maize	14	Crude protein (%) (by analysis)	13.5
Tic beans	9	Approx. starch equivalent (%)	68
Grass meal (ground)	9	Approx. digestible crude protein (%)	11
Soya bean meal	9		
Dairy cubes (balanced)	9		
Locust bean meal	5		

Roughages		
	Dried grass	Silage
Dry matter (%)	89.2	22.1
Crude protein in dry matter (%)	11.0 (Range 8.4 - 12.9)	12.3
Approx. starch equivalent	56	50
Approx. digestible crude protein	6	7

The values of the dried grass and the silage were calculated using the regression equations of Watson (1939) or by taking his values. The silage was fed after the evening milking, and the dried grass at 7.00 a.m. and at 12 noon.

The concentrates for each cow were individually weighed every day whereas the silage and dried grass were weighed in bulk and then divided carefully between the twelve cows. All the foods were sampled weekly to determine the dry matter and crude protein content.

The silage fed during the experiment had been made from grass cut at the long leafy stage from leys which had received heavy dressings of balanced artificial fertilizers. It was made from July 7th-18th, 1956, had no preservative added to it and contained virtually no clover. The finished product was well made, had a pleasant smell and was very palatable to the stock. The dried grass was made from similar leys between July 26th and September 25th, but was cut at a leafier stage.

On the day after calving 10 lb. of concentrates were offered to each animal. This was increased to 16 lb. on the 15th day after calving, and thereafter the weight of concentrates was adjusted weekly on the basis of 4 lb. of concentrates per 10 lb. of milk. After the cows had access to the pasture, the level of concentrate feeding was progressively lowered until on April 29th it was finally stopped.

Housing and weighing

The cows were housed throughout the experiment in a double-stall byre but were allowed out each day from 10.30 a.m. to 12 noon into a bare paddock. They were weighed on one morning each week at approximately 12 noon. Both the cow and the calf were also weighed within 12 hours after calving.

Milking

After it had been weighed the calf was separated from the cow which was then milked twice a day. All yields were recorded to the nearest $\frac{1}{4}$ lb.

Blood samples and analysis

A sample of blood was taken from each cow once per week for a minimum of 6 weeks before calving, and for 3 to 9 weeks after calving depending upon the length of time between calving and the beginning of the grazing season. The total acetone bodies in each sample were determined by the method of Thin & Robertson (1952).

Other analyses and tests

After March 26th a sample of milk from each cow was subjected each day to Rothera's test, and according to the colour reaction obtained it was graded +ve, —ve or doubtful (+/—ve). From the same date once per week, the levels of acetone in samples of mixed evening and morning milk were also determined by the method of Thin & Robertson (1952).

Treatment of cows

If a cow developed severe clinical symptoms of ketosis she was given one or more doses of 400 ml. of glucose solution (50%) intravenously, and 4-6 oz. of sodium propionate as a drench for three consecutive days and her appetite tempted by offering her mangolds and hay.

XUM

Grazing

As will be reported later in this paper, the cows in both groups were first allowed access to fresh grass between March 26th and April 9th to investigate the effect of this food on the ketosis. On the first day the animals were on the grass for only 1 hour, but the time was increased daily until on March 31st they were on the grass for 5 hours a day. This was further increased to 7 hours on April 3rd, until finally on April 16th the animals were out at pasture both day and night. The grass offered to the cows was a mixture of Italian and Perennial Ryegrass sown the previous year and had received 2 cwt./acre of 'Nitro-Chalk' (15.5% nitrogen) on March 6th, to assist the production of an 'early bite'. The grass was from 6-9 inches high and very leafy when grazing started, and it contained 24.6% crude protein in its dry matter when sampled on March 27th. The experimental cows were folded carefully over the grass using an electric fence and were made to graze the sward fairly closely.

Dates

The experiment lasted from January 7th to April 26th, 1957.

Results

Incidence of ketosis and blood values

The values for the content of ketone bodies in the blood of the cows expressed as mg. acetone per 100 ml. of blood are given in full in Table 3. Two cows, No. 5 in the high level group, and No. 12 in the low level group are grouped separately in the table because they were the last two animals to calve, and were allowed out to grass 3 days after calving. One blood sample was, however, taken from each of them after they had calved but before they went out to pasture.

It will be observed from Table 3 that before calving the level of ketone bodies

TABLE 3

Content of ketone bodies in the blood of the cows in the two groups. (mg. acetone per 100 ml.)

Cow number	Days before calving						Days after calving								
	42–36	35–29	28–22	21–15	14–8	7–0	0–7	8–14	15–21	22–28	29–35	36–42	43–49	50–56	57–63
High level of concentrates															
1	2.9	2.2	3.9	2.5	3.9	2.5	14.6	29.3	20.0	38.5	59.6*	45.0	3.4	6.1	3.7
2	5.2	4.0	3.8	4.1	2.2	4.0	23.8	15.7	32.5	47.1*	27.6	23.8	5.7	4.5	
3	4.5	4.6	2.5	2.9	11.3	2.5	4.1	13.0	11.4*	23.3	4.0	2.2	2.9		
4	5.0	2.9	4.2	4.2	3.5	1.6	44.7	37.9*	34.7	4.0	3.5	4.5			
5	4.7	2.2	17.3	3.3	2.2	3.3	9.2	52.5*	3.7	2.9	2.7				
Mean	4.5	3.2	6.3	3.4	4.6	2.8	19.3	29.7							
Low level of concentrates															
7	–	2.7	–	3.9	3.3	3.9	9.2	11.9	14.6	7.7	33.0	56.6*	10.8	3.4	1.1
8	3.3	2.8	3.5	3.5	3.5	2.9	6.5	42.2	28.1	39.5	100.0*	62.3	23.8	6.8	3.4
9	4.5	4.7	5.0	3.4	4.8	2.2	2.2	21.1	22.7*	31.4	14.0	5.7	3.4		
10	5.2	5.4	4.1	3.7	5.2	4.0	6.9	24.9	40.1*	41.7	15.2	2.3	4.5		
11	3.3	2.8	4.2	4.2	2.2	2.5	6.9	23.8	18.4*	6.0	3.3				
Mean	4.1	3.7	4.2	3.7	3.8	3.1	6.3	24.8	24.8						
High 5	4.2	4.5	2.2	2.7	4.1	6.5	7.2*	7.2	5.7						
Low 12	4.2	3.9	2.5	4.5	2.7	8.8	4.9*	4.6	3.4						

* Cows out to grass for first time.

in the blood from both groups was reasonably constant and that apart from two individual values it was at a low level. The mean value for the high level group (5 cows) was 4.1 mg., and for the low level group (5 cows) 3.8 mg. The lowest mean

values in both groups were found in the period 7-0 days before calving, but apart from that, there was no definite trend in the values.

Immediately after calving the ketone values started to increase, and as seen in Table 3, the mean values were 29.7 and 24.8 mg. respectively for the high and low level groups (5 cows per group) in the period 8-14 days after calving. There was considerable variation between the values for the individual animals after 15 days but this can doubtless be attributed to the fact that some of the cows had access to grass after that time while others did not. It would not, therefore, be useful to quote means in Table 3 for the periods after 15 days from calving. A study of the results from individual animals shows clearly that there was always a marked rise in ketone bodies in the blood soon after calving and that every animal, to a lesser or greater degree suffered from ketosis as determined by this means, except for cows 5 and 12 which went out to grass very soon after calving. In order to give an indication of the degree of ketosis in the animals in the two groups, they have been sub-divided arbitrarily into groups according to the method of Thin & Robertson (1953). This is based on the level of total ketones in the blood and is as follows: —

	mg. acetone/100 ml. blood	
Group A	> 60	severe clinical cases
Group B	59-30	milder clinical cases
Group C	29-10	sub-clinical cases

The results of this grouping are shown in Table 4 from which it can be seen that the incidence of ketosis and its severity were identical in both groups. The results for cows 5 and 12 have been excluded from this table since these two were put out to

TABLE 4

The number of cows grouped according to the maximum value of ketone bodies in the blood.

		Group A (ketone bodies (mg. acetone/100 ml.) >60)	Group B (ketone bodies (mg. acetone/100 ml.) 59-30)	Group C (ketone bodies (mg. acetone/100 ml.) 29-10)
		No. of cows		
Level of concentrates	High	1	3	1
	Low	1	3	1

pasture so soon after calving. The striking difference between these two cows and the other ten, can only be attributed to the fact that they went out to grass immediately.

Effect of turning the cows out to grass

The time after calving at which the individual cows had access to pasture is indicated in Table 3. This varied from 0-7 days with cows 5 and 12 to over 42 days with cow 7. At the time at which ten of the cows were turned out to pasture the general level of ketosis was high and only the severest cases were responding to the glucose and propionate treatment. Thus, in order to follow the effect of the grass even more closely, milk samples were examined daily by Rothera's test.

The changes in the blood ketone values can be seen for the individual cows in Table 3, and the results are summarised in Table 5. It will be seen that the high values of 41.7 and 43.6 mg. for the high and low level groups respectively decreased at each successive sampling, until, after a period of 15-21 days, they were back to what may be classed as normal values. The trends in both groups were very similar.

XUM

A similar trend was evident with the acetone levels of the milk. Six days before turning the cows out to pasture and for 3 days afterwards, the values for individual milks varied from 0.3 to 18.8 mg. with averages of 7.7 and 4.7 for the high and low level groups respectively. Eighteen days after the grass feeding began seven of the ten samples were negative and the remaining three were 1.4, 1.5, and 2.8 mg. This last value was a sample from cow 8 which had had the severest clinical symptoms and which took the longest time to recover her normal health. (This cow consequently yielded 948 gallons in a lactation of 312 days). Using the grouping procedure for milks similar to that used above for bloods (Thin & Robertson, 1953), no difference was found between the high and low level groups.

When using this method of grouping the results, it was found that the values for the acetone levels in the milk always tended to underestimate the severity of the ketosis symptoms when compared with the blood values. In every case the blood values were a surer guide when classifying and grouping the severity of the disease.

TABLE 5

Content of ketone bodies in the blood before and after the cows went out to grass. (5 cows per group.)

Days		Before 0-7	After access to pasture 0-7	8-14	15-21
		(mg. acetone per 100 ml.)			
Level of concentrates	High	41.7	26.9	7.6	4.0
	Low	43.6	34.4	11.9	4.5
Mean of both groups		42.6	30.7	9.8	4.3

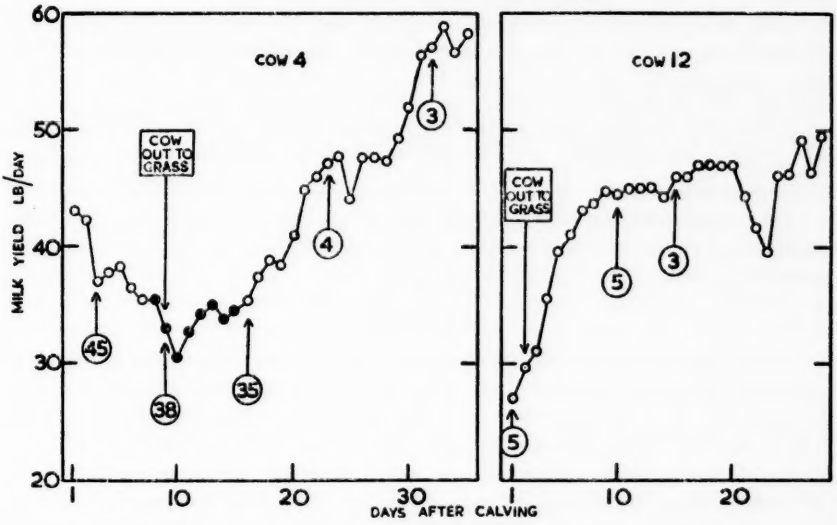
The results of Rothera's test, the only subjective qualitative test used in the trial, were in close agreement with both the blood ketone and the milk acetone results and showed a similarly steady decline in the number of cows affected, and in the severity of the ketosis as the grazing period advanced. The number of positive daily tests for the milk varied from thirteen for the worst affected cow (No. 8) to only one where the symptoms were much less severe. The mean number of days on which positive results were obtained was 6.2 on the high level treatment and 5.2 on the low.

Milk yield

As can be imagined, the high incidence of clinical and sub-clinical ketosis interfered greatly with milk production and too much importance should not, therefore, be attached to the mean daily milk yield of the two groups, but excluding cows 5 and 12 the mean daily yield over the first 56 days of the lactation was 45 lb. for the high level group and 40 lb. for the low level group. Of greater interest is the shape of the lactation curve just after calving. With each cow, as the level of blood ketones increased, milk production decreased, and, instead of the normal lactation curve with a peak a few weeks after calving, the reverse often happened and milk production fell a week or so after calving. This is well illustrated in Fig. 1 in which the milk yield of cow 4 is plotted. The milk yield of this cow continued to fall even when she was first out at grass, but it slowly recovered as her blood ketones decreased and it finally reached a peak yield of 58 lb./day. The lactation curve can be compared with that of cow 12 which had no ketosis and showed a normal rise in yield after calving. The effect on yield of going out to grass was quite spectacular in both groups. Within 15

FIG. 1

Daily Milk Yields of Cows 4 and 12. Figures in circles are the values of the ketone bodies in the blood expressed as mg. acetone per 100 ml.
● indicates positive Rothera's test in the milk.
Cow 4 out to grass 9 days after calving.
Cow 12 out to grass 2 days after calving.



days of being out at pasture the high level group had increased in yield by 12.6 lb. per cow per day and the low level group by 11.3 lb.

The loss of milk as a result of the ketosis has been calculated by measuring the area between the recorded lactation curve and an assumed curve drawn from the starting point to the peak yield, and bearing in mind the previous year's lactation. Where there was doubt as to whether the curve should be in a lower or higher position, it was put at the lower one so that the loss of milk would not be overestimated. The amount of milk lost by each cow before she gained her normal state of health varied from a minimum of only 4% with cow 3 to a maximum of 21% with cow 8. The average loss for the high level group was 14% and for the low level group, 16%.

Milk quality

The average butter-fat content of the milk from the time of calving until the ketone content of the blood was normal, averaged 5.1% for the high level group and 5.0% for the low level group. The solids-not-fat values calculated in a similar way were 9.2% and 8.9% for the high and low level groups respectively. No relationship was found between the acetone content of the milk and its fat and solids-not-fat content.

Liveweights

The general pattern of liveweight change in the two groups of cows was surprisingly similar. In both groups the mean daily liveweight increase was 1.4 lb. during the last 28 days before calving whilst at calving the total decrease in weight

XUM

(calf plus foetal fluids, etc.) was 120 lb. for the high level group and 122 lb. for the other. After calving the high level group lost an average of 122 lb. in 28 days compared with the low level group that lost 105 lb. in the same period. After a further 7 days, by which time most of the cows had been out to grass, both groups began to gain a little weight.

The mean birthweight of the calves on the high and low level treatments were 83 and 73 lb. respectively, but this difference was not statistically significant.

Other metabolic diseases

Two cows in each group had milk fever at the time of calving and were treated with calcium borogluconate. All four responded to the treatment.

Concentrate consumption

The mean daily consumption of the concentrate mixture from the day of calving until April 29th, when it was stopped, was 11.3 lb. per cow for the high level group and 11.9 lb. per cow for the low level group.

Discussion

From the results of this trial it is clear that, on the rations fed, the level of 'steaming-up' before calving had no effect on the incidence and severity of ketosis. This result is in broad agreement with that reported by Hathaway, Brakel, Tyznik & Kaeser (1957) who found no difference in the incidence of ketosis or in blood ketone levels after calving in two groups of cows which were fed either 4 lb. or 12-14 lb. of concentrates daily for the last 14 days before calving. The 'steaming-up' of cows with concentrates is known to have a beneficial effect on the subsequent lactation (Blaxter, 1944) and is a widely recommended practice. In this trial milk yields were slightly higher on the higher 'steaming-up' ration, but the high incidence of ketosis in both groups rather masked this fact. In this experiment the reasons for the high incidence of ketosis appear to have been associated with factors other than the level of concentrate feeding before calving.

The blood ketone levels of the twelve cows before calving averaged 4.0 +/—2.2 mg., which, although low by the classification of Thin & Robertson (1953), is higher than the mean values of 2.5 +/—1.0., and 2.0 +/—0.9, found by Horrocks & Paterson (1957) in cows with a history of previous ketosis and in normal cows. Slight differences in the analytical technique may explain part of the difference but even so it would seem that even before calving the blood ketone values were somewhat higher than normal. After calving, the values found in our trial were typical of the results quoted by Thin & Robertson (1953) for cows with ketosis.

The reason for the high values and the severe level of ketosis can only be a matter of speculation. Brouwer & Dijkstra (1938) showed that the feeding of grass silage of the butyric acid type caused an increase in the ketone bodies in the urine of cattle, compared with the values obtained on a ration containing hay, whilst more recently, Adler (1956) has reported that in a herd where 120 lb. of silage was fed per day there was a widespread positive reaction to a milk test for acetone bodies. This author arrived at no definite conclusions but considered that the intake of silage may have been the 'last straw'. In our trial only 28 lb. of silage were fed daily and this may or may not have been a contributing factor. The remainder of the basal ration

XUM

consisted of another grassland conservation product, dried grass, and there was also 9% of ground dried grass in the concentrate mixture (Table 2). Barnett & Reid (1957) have shown that acetic acid predominated when dried grass was acted upon by rumen liquor in an artificial rumen, and in sheep, a highly fibrous ration will produce a predominantly acetic acid fermentation (Armstrong, Blaxter & Graham, 1957). It may have been, therefore, that the rations in the present trial resulted in a relative deficiency of propionic acid in the rumen.

The value of fresh spring grass in reducing the ketosis was very apparent and is in complete agreement with the results of Knodt, Shaw & White (1942) and of Shaw, Powell & White (1942). The former workers noted a sharp decline in acetone bodies after only 1 day of pasture feeding, and after 16 days the blood acetone bodies had decreased by 53%. In our trial the decrease after 15-21 days at pasture was 90%. Allcroft (1947) from an analysis of ketosis records concluded that access to pasture had little effect on the incidence of ketosis. The 33 records were made between December and April, and it is more than possible that the 'pasture' was, in fact, a bare grass field and was supplying a mere fraction of the animals' daily nutrient intake. The pasture grass in our trial had a marked anti-ketogenic effect even when silage was being fed at the same time. The value of an 'early bite' of good grass is well known as a means of reducing the cost of feeding the dairy cow (Hamilton, 1952) but it can now be further recommended as a safe and effective method of preventing or eliminating ketosis. In late winter ketosis is generally at its peak and the value of this early grass could be very great.

The loss in milk that appeared to occur as a result of ketosis in the present trial averaged 15%. This, it is considered, is a conservative value but it could make a difference of at least 50-75 gallons in a complete lactation. The loss of milk occurred during the first few weeks, which is a critical time in the cow's lactation, and, as shown by Blaxter (1956), the longer milk yield is allowed to remain at an abnormally low level, the poorer is the chance of re-establishing it at its former level.

Only 12 cows were used in this experiment, and as in the work of Horrocks & Paterson (1957) there was a considerable variation between individual cows. In spite of this, however, the results are clear. Further work on other aspects of the ketosis problem is planned.

Summary

1. Twelve Ayrshire cows aged from 3¼ to 9¾ years were divided into two balanced groups based on age, liveweight, length of dry period and date of calving.

2. All the cows were fed 28 lb. grass silage and 8-12 lb. dried grass per day before and after calving.

3. One group, the high level group, was fed 252 lb. concentrates per cow as a 'steaming-up' ration in the 6 weeks before calving. The other group, the low level group, received 126 lb. in the same period. After calving both groups were fed 4 lb. of concentrates per 10 lb. milk produced.

4. In each group after calving there were five cases of ketosis distributed as follows: one clinical severe, three clinical and one sub-clinical.

XUM

5. The mean levels of ketone bodies in the blood expressed as mg. acetone per 100 ml. were 4.1 and 3.8 in the high and low level groups respectively before calving, and 29.7 and 24.8 8-14 days after calving.

6. When the cattle were allowed to graze a pasture of early growing grasses, the mean value of blood ketones for both groups fell progressively from 42.3 mg. acetone to 4.4 mg. 15-21 days later. Two cows which were allowed to graze 3 days after calving did not develop any symptoms of ketosis and their blood values were within the normal range.

7. Liveweight changes in both groups were very similar.

8. The average butter-fat and solids-not-fat contents of the milk in the high level group were 5.1% and 9.2% respectively. Corresponding values for the low group were 5.0% and 8.9%.

9. It was calculated that on average there was a loss of 15% of milk yield in the early part of the lactation when the animals were suffering from ketosis.

The authors thank Mr. J. N. Watson for assistance with the animals and Dr. R. Waite for the analyses of the milk samples.

REFERENCES

Adler, J. H. (1956): *Cornell Vet., 46,* 446.
Allcroft, W. M. (1947): *Vet. J., 103,* 2.
Armstrong, D. G., Blaxter, K. L. & Graham, N. McC. (1957): *Proc. Brit. Soc. Anim. Prod.* 1957, 3.
Barnett, A. J. G. & Reid, R. L. (1957): *Biochem. J., 65,* 13.
Blaxter, K. L. (1944): *J. agric. Sci., 34,* 27.
Blaxter, K. L. (1956): *Proc. Brit. Soc. Anim. Prod.* 1956, 3.
Brouwer, E. & Dijkstra, N. D. (1938): *J. agric. Sci., 28,* 695.
Hamilton, R. A. (1952): *J.Fmrs'Clubs,Lond.,* 4, 3.
Hathaway, H. D., Brakel, W. J., Tyznik, W. J. & Kaeser, H. E. (1957): *J. Dairy Sci., 40,* 616.
Horrocks, D. & Paterson, J. Y. F. (1957): *J. comp. Path, 67,* 331.
Knodt, C. B., Shaw, J. C. & White, G. C. (1942): *J. Dairy Sci., 35,* 837.
Shaw, J. C., Powell, R. C. & White, G. C. (1942): *J. Amer. vet. med. Assoc., 100,* 473.
Thin, C. & Robertson, A. (1952): *Biochem., J., 51,* 218.
Thin, C. & Robertson, A. (1953): *J. comp. Path., 63,* 184.
Woodman, H. E. (1954): *Bull. Minist. Agric.,* Lond., No. 48.

THE EFFECT OF ANTIBIOTIC SUPPLEMENTS ON THE FAECAL STREPTOCOCCI (LANCEFIELD GROUP D) OF POULTRY

By ELLA M. BARNES

Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research.

There has been considerable discussion on whether the continuous use of antibiotic supplements for growth promotion in pigs and poultry leads to the development of a resistant microbal population in the gut. Most concern has been expressed with regard to the possible development of a resistant pathogenic flora which may directly or indirectly be the cause of disease in the animals. More recently the presence or absence of resistant faecal organisms has taken on a new significance because of the permitted use in several countries of the same antibiotics (chlortetracycline* and oxytetracycline†) for poultry and fish preservation, and their proposed use for the preservation of meat at warm temperatures. (Ginsberg, Hill & Grieve, 1957). The present paper is concerned mainly with this particular aspect of the situation.

A survey has been made of the faecal streptococci (Lancefield group D) normally present in the intestines of poultry and the effect of chlortetracycline supplements on these organisms. These latter experiments were carried out in collaboration with Dr. R. F. Gordon of the Houghton Poultry Research Station who was extending the studies of Smith and Crabb (1957) on resistant populations, by following changes in the antibiotic resistance of the coliforms throughout the life of the bird and will be reporting his results elsewhere (Gordon & Tucker, 1958).

Most of the studies on the faecal streptococci were made on birds at 10-12 weeks of age because this was the time that they would, as ' broilers ', be killed and processed and the condition of the faecal population be critical in relation to spoilage of antibiotic treated poultry as well as to food borne diseases of faecal origin.

Materials and Methods

Rearing and feeding of birds

Birds from a number of localities were examined in order to estimate the typical population of faecal streptococci. They had been reared throughout the whole of their life on various standard broiler diets without any added antibiotic.

To determine the effect of antibiotic feeding on the gut flora, three groups of birds were reared under identical conditions on a standard broiler diet, at the Houghton Poultry Research Station. One group was fed on the control ration without antibiotic, a second had the control ration with 25 p.p.m. chlortetracycline throughout life, whilst the third group was fed for the first 6 weeks on the control ration, from 6-8 weeks on the control ration with 400 p.p.m. chlortetracycline, from 8-10 weeks on the control ration again and finally from 10-12 weeks on the control ration with 400 p.p.m. chlortetracycline.

* The trade name for chlortetracycline is 'Aureomycin'.
† The trade name for oxytetracycline is 'Terramycin'.

XUM

Faecal and caecal samples

The faecal samples were collected by placing large sheets of filter papers overnight in each cage of 50 birds. The samples were bulked, well mixed and then aliquots removed for antibiotic assay and bacteriological examination. Faecal samples may be unsatisfactory as they are not always representative of a majority of the birds and the numbers and types of organisms may alter during the interval between voiding and collection. Confirmatory analyses were made on individual caecal samples when the birds were killed. The whole contents from either one or both caeca of freshly killed birds were squeezed into sterile weighed beakers. After mixing thoroughly, samples were taken for analysis, as follows.

Chlortetracycline assay

The method used was adapted from the microbiological assay of Abbey *et al.* (1956-7) for determining chlortetracycline in tissues. The initial extract was prepared by shaking 1 g. of faeces or caecal contents with 4 ml acid acetone, pH 4.2 in a 1 oz. bottle containing glass beads and then centrifuging. The activity of each sample was related to that of a standard solution prepared by the method of Grove & Randall (1955) but finally diluted in the acid acetone to be comparable with the faecal and caecal extracts. On all occasions control samples of acid acetone, caecal contents etc. were tested at the same time on the assay plates. It was only very occasionally that the control gut samples showed a slight trace of activity (equivalent to < 0.1 p.p.m.).

Bacteriological analysis

One gramme of the sample was weighed into a wide necked 1 oz. bottle containing 15 glass beads and shaken mechanically with 9 ml of quarter strength Ringer's solution. Decimal dilutions of the sample were prepared and 0.025 ml of each dilution was spread over one-quarter of Petri dishes containing the required medium.

Total viable counts

These were made on Hartley's digest agar and the plates incubated at 37°C for 1 day. Experience has shown that neither the lactobacilli nor the clostridia grow under the conditions used, so this count consisted mainly of faecal streptococci, coliforms and other Gram—ve organisms. To determine the numbers of chlortetracycline resistant organisms a freshly prepared aqueous solution of chlortetracycline was incorporated in the cooled agar immediately before pouring the plates, to give final concentrations of 10 and 50 p.p.m.

Faecal streptococci

The method used for the enumeration and isolation of the faecal streptococci was that of Barnes (1956). The composition of the thallous acetate tetrazolium agar was—peptone (Evan's) 1%, Lab-Lemco 1%, glucose 1%, thallous acetate 0.1%, 2 : 3 : 5-triphenyltetrazolium chloride 0.01%, agar (New Zealand) 1.4%; pH 6.0. The solution containing the peptone and Lab-Lemco was adjusted to pH 6.0 and autoclaved with the agar in 95 ml. lots at 15 lb/in^2 for 20 min. Then 5 ml. of sterile 20% glucose solution were added together with 1 ml. of a 1% solution of the tetrazolium salt which had been sterilised by steaming for 30 min., and 2 ml. of a 5% solution of thallous acetate.

The plates were incubated for 24 hrs. at 37°C. The streptococcal colonies could be divided into two groups (1) White or very pale pink colonies, characteristic of the group D streptococci with low reducing powers—*Streptococcus faecium, Streptococcus durans,* some strains of *Streptococcus bovis* and a number of unclassified strains. (2) Colonies with a deep red centre and narrow white periphery, characteristic of *Streptococcus faecalis* and its variants *zymogenes* and *liquefaciens.* An occasional background haze of minute colonies was disregarded. The colonies were picked into thallous acetate broth, purified and identified by the methods used by Barnes, Ingram & Ingram (1956).

Sensitivity to chlortetracycline and oxytetracycline

Tests showed that it was not possible to count faecal streptococci on thallous acetate tetrazolium agar containing the antibiotic as there seemed to be an additional inhibitory effect. To determine the sensitivity of the faecal streptococci, overnight broth cultures of the organisms were streaked on Hartley's digest agar (pH 7.2) containing 0, 10, 50 or 200 p.p.m. chlortetracycline or oxytetracycline. The plates were then incubated overnight at 37°C and positive growth recorded. It must be emphasised that the results so obtained are only of comparative value, the activity of these antibiotics being related to many factors such as the pH and composition of the medium, inoculum size etc. For accurate comparisons about 60 strains from both control and antibiotic fed birds were tested at the same time under identical conditions.

Results

Faecal streptococci in the intestines of birds fed without antibiotic supplements

The poultry examined during the past 2 years were taken from at least four different localities. Most of the studies were made on the caecal flora as the caecum is one of the largest reservoirs of organisms in the gut at death. The numbers of faecal streptococci in the caecum varied between 10^4 and 10^7/g. and they were always outnumbered by both the lactobacilli and coliform organisms.

The method of counting used (see above) enabled an immediate differentiation to be made between *Streptococcus faecalis* and its variants *zymogenes* and *liquefaciens* which reduced tetrazolium, and *Strep. faecium* and other species which did not reduce tetrazolium. The two commonest species of faecal streptococci present were found to be *Strep. faecalis* var. *liquefaciens* and *Strep. faecium.* Of 70 red centred colonies examined from 50 birds 67 were identified in detail as *Strep. faecalis* var. *liquefaciens,* 2 as *Strep. faecalis* and 1 as *Strep. faecalis* var. *zymogenes.* Of 45 white colonies isolated 39 were identified as *Strep. faecium.*

Resistance to antibiotics

The sensitivity to chlortetracycline and oxytetracycline of a number of the strains isolated was determined on nutrient agar containing 0, 10, 50 and 200 p.p.m. antibiotic (see above). The results with chlortetracycline, given in Table 1, show that although the majority of strains of *Strep. faecalis* var. *liquefaciens* were inhibited by 10 p.p.m. chlortetracycline, about 16% were able to grow on 50 p.p.m. Of the strains of *Strep. faecium* tested about 60% were resistant to 50 p.p.m. The results with

XUM

TABLE 1

The sensitivity to chlortetracycline of faecal streptococci isolated from the intestines of birds reared without antibiotic supplements.

Species	Number of strains	Growth on nutrient agar containing chlortetracycline p.p.m. 0	10	50	200
Strep.faecalis var.liquefaciens	24	+	-	-	-
	5	+	+	+	-
Strep.faecalis	1	+	-	-	-
Strep.faecium	8	+	-	-	-
	12	+	+	+	-
Unclassified	2	+	-	-	-

oxytetracycline are not included in the Table as they were very similar except that 25% of the strains of *Strep. faecium* were able to grow at the 200 p.p.m. level.

Effects of chlortetracycline in the feed

For the first part of this investigation the control group of birds was compared with birds fed on 25 p.p.m. chlortetracycline supplements throughout life and with birds given two intermittent fortnightly feeds of 400 p.p.m. (see above). Bulk faeces samples were collected at weekly intervals from the 5th-12th weeks for all three groups and showed little difference in total numbers of faecal streptococci (Table 2). In all

TABLE 2

The chlortetracycline content and numbers of faecal streptococci in faeces of birds fed on rations containing 0.25 and 400 ppm. chlortetracycline.

Time in weeks	Control group		Ration + 25 ppm. chlortetracycline			Ration + 400 ppm. chlortetracycline		
	Red colonies/	White colonies*	Red colonies	White colonies	C.T.C.* p.p.m.	Red colonies	White colonies	C.T.C.* p.p.m.
5	-&	1.3×10^7	-	1.4×10^7	0.35	-	1.7×10^7	not tested
6	-	2.1×10^6	-	4.1×10^6	0.6	-	1.4×10^7	not tested
7	6×10^5	5.8×10^6	-	4.8×10^6	2.9	-	1.2×10^7	26.0
8	-	4×10^6	-	1.8×10^6	2.0	-	4.1×10^7	31.0
9	-	7.9×10^6	-	1.4×10^7	1.7	-	2.4×10^7	tr.
10	1.8×10^6	1.6×10^7	1.5×10^6	7×10^7	1.0	1.2×10^6	3.5×10^7	0.5
11	3.4×10^5	1.2×10^7	8.6×10^5	1.2×10^8	1.0	8×10^4	3.4×10^7	23.5
12	4.1×10^7	1.4×10^6	3.8×10^7	4.6×10^8	not tested	6.9×10^7	4×10^7	not tested

/ Red colonies - Strep. faecalis and Strep. faecalis var liquefaciens types

*White colonies - Strep. faecium types

& the sign - indicates that red centred colonies were outnumbered 100:1 by white ones.

* C.T.C. = chlortetracycline.

cases there was a preponderance of *Strep. faecium* types for the first 9 weeks, but by the 12th week *Strep. faecalis* and variants were being voided in large numbers. Chlortetracycline assays showed that the concentration of chlortetracycline in the faeces from the birds fed 25 p.p.m. supplement varied between 0.3 and 3 p.p.m. whilst in the samples taken in the weeks following the commencement of the 400 p.p.m. supplement the highest concentration found was 31 p.p.m. (Table 2). The concentration fell again immediately following the withdrawal of the 400 p.p.m. supplement (9th and 10th weeks).

After the 8th week when the 3rd group had been on the 400 p.p.m. chlortetracycline supplement for 2 weeks the resistance of the faecal population was determined by counting on nutrient agar containing 10 and 50 p.p.m. chlortetracycline. The

TABLE 3

The effect of chlortetracycline in the feed on the resistance of the gut flora of 8 week old birds.

Ration	Total count* at 37°C micro-organisms/g faeces		
	Nutrient agar	Nutrient agar + 10 ppm. chlortetracycline	Nutrient agar + 50 ppm. chlortetracycline
Normal diet (1)	1.7×10^7 (8.2×10^6) †	$< 4 \times 10^4$	$< 4 \times 10^4$
Normal diet (2)	4.3×10^6 (1.1×10^6)	$< 4 \times 10^4$	$< 4 \times 10^4$
25 ppm. chlortetracycline throughout life	1.5×10^8 (3.6×10^6)	1.4×10^8 (about 3×10^6)	9.8×10^7 (4×10^6)
400 ppm. chlortetracycline for 2 previous weeks	4.2×10^8 (3.6×10^7)	4.1×10^8 (4.2×10^7)	3.6×10^8 (3.3×10^7)

* Total count consists of all organisms except lactobacilli and clostridia (Coliforms and faecal streptococci usually predominate).

† Faecal streptococci.

results in Table 3 show that in the birds which had been fed with 400 p.p.m. chlortetracycline the organisms were completely resistant to 50 p.p.m. chlortetracycline, whilst the organisms from the control birds were inhibited by as little as 10 p.p.m. More than half of the population in the birds fed on 25 p.p.m. was resistant to the 50 p.p.m. chlortetracycline.

Twelve birds in each group were killed after 12 weeks. A detailed examination was made of the caecal contents from 3 in each group, the others were used for

XUM

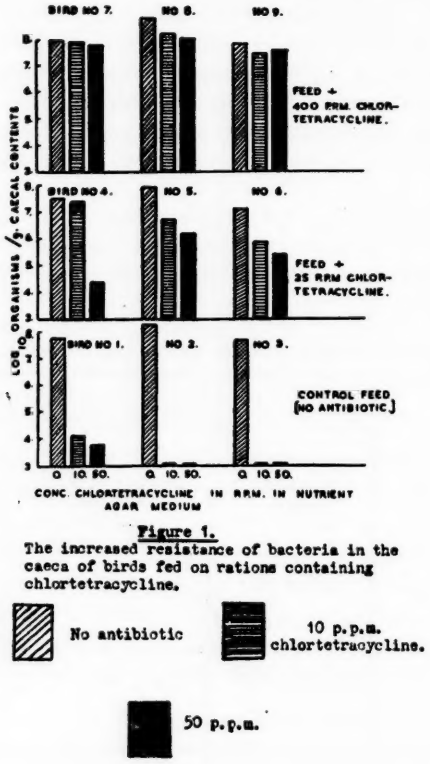


Figure 1.
The increased resistance of bacteria in the caeca of birds fed on rations containing chlortetracycline.

confirmatory checks. The results in Figure 1 show that in the controls less than 1 in 10,000 organisms was able to grow in the presence of 50 p.p.m. chlortetracycline, whilst in the birds fed on 400 p.p.m. chlortetracycline more than 60% of the population was resistant. The caecal population of the birds fed even on 25 p.p.m. throughout life also showed a high degree of resistance. The concentration of chlortetracycline in the caecum of birds fed on 25 p.p.m. was about 0.5 p.p.m. whilst the caecal contents of three of the 400 p.p.m. fed birds contained 3.8, 4.7 and 2.5 p.p.m.

The corresponding numbers and types of faecal streptococci present in the three groups of birds are shown in Figure 2. Although only 3 birds in each group are recorded in the table, the results were confirmed on the other 9 from each group. In the control birds *Strep. faecium* strains predominated and of 17 strains isolated from the highest dilutions all were inhibited by 10 p.p.m. chlortetracycline. The *Strep. faecalis* and variant types were present in very small numbers. Of 20 strains isolated 16 were *Strep. faecalis* var. *liquefaciens* and 4 were *Strep. faecalis*. All were inhibited by 10 p.p.m. chlortetracycline except 1 strain of *Strep. faecalis* var. *liquefaciens* which grew at 50 p.p.m. (Table 4).

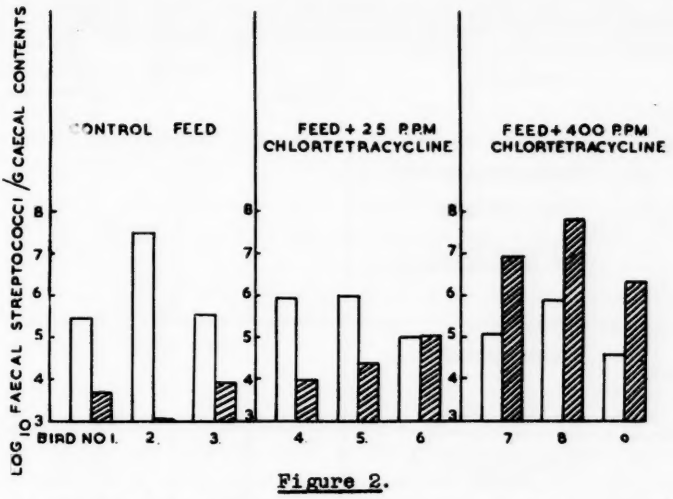

LOG10 FAECAL STREPTOCOCCI /G CAECAL CONTENTS
CONTROL FEED
FEED + 25 P.P.M CHLORTETRACYCLINE
FEED + 400 P.P.M CHLORTETRACYCLINE
BIRD NO 1. 2. 3. 4. 5. 6. 7. 8. 9.

Figure 2.

A comparison of faecal streptococci in the caeca of 12 week old birds fed on rations containing chlortetracycline.

Strep. faecium types

Strep. faecalis
Strep. faecalis var. liquefaciens

In the caeca from birds fed on 400 p.p.m. chlortetracycline *Strep. faecium* was still present but was considerably outnumbered by a strain of *Strep. faecalis*. This organism was different from the strains found in the control birds: it was a very strong reducer of tetrazolium colonies and had a characteristic dark red colour. It failed to liquefy gelatin, and was resistant to 200 p.p.m. chlortetracycline. Selected strains also only reacted with difficulty with the Lancefield group D antiserum, the concentrating methods of Shattock (1949) being necessary. No strains of *Strep. faecalis* var. *liquefaciens* were found in these birds. The *Strep. faecium* strains in the birds given the 400 p.p.m. supplement were also much more resistant than those in the control birds (Table 4). An apparently identical antibiotic resistant strain of *Strep. faecalis* was seen in birds fed on 25 p.p.m. antibiotic; of the 39 cultures isolated (from 6 birds) all were resistant to 200 p.p.m. chlortetracycline, and again no organisms similar to *Strep. faecalis* var. *liquefaciens* were found (Table 4).

Effect of withdrawing the antibiotic supplement

To determine whether the typical resistant population of faecal streptococci in the caeca of the antibiotic fed birds would remain dominant in the absence of the antibiotic, some of the birds which had been fed on the rations containing 400 p.p.m. chlortetracycline were fed for a further 3 months on the control diet without anti-

XUM

TABLE 4
Chlortetracycline resistance of faecal streptococci isolated from the caeca of chickens at 12 weeks.

Group	Organism	Nos. of strains	Growth on agar containing chlortetracycline p.p.m. 0	10	50	200
Control	*Strep. faecium* types	17	+	-	-	-
	Strep. faecalis	4	+	-	-	-
	Strep. faecalis var. *liquefaciens*	15	+	-	-	-
	*	1	+	+	+	-
Feed + 25 p.p.m.	*Strep. faecium*		NOT TESTED			
	Strep. faecalis	39	+	+	+	+
Feed + 400 p.p.m. 6–8 weeks 10–12 weeks	*Strep. faecium* types	9	+	+	+	-
	Strep. faecalis	18	+	+	+	+

† No detailed identification was made of these organisms.

TABLE 5
The faecal streptococci in the faeces of birds 3 months after the last antibiotic feed.

Group	White colonies *Strep. faecium* types organisms/g	Red centred colonies *Strep. faecalis* and variants organisms/g
Control birds (faecal droppings)	* 5.3×10^5	1.2×10^7
	$< 4 \times 10^4$	8.4×10^6
Birds 3 months after the last 400 p.p.m. supplement (faecal droppings)	9.2×10^4	5.4×10^6
	$< 4 \times 10^4$	1.7×10^7
(caecal droppings)	$< 4 \times 10^3$	2.8×10^4
	6.8×10^5	6.4×10^5
	$< 4 \times 10^3$	6.1×10^6

* Each count was from a bulk sample from 25 birds.

biotics and compared with some of the control birds which had never received the antibiotic supplement. Bulk faecal samples were collected and caecal droppings were also examined.

The counts of faecal streptococci shown in Table 5 indicate that the red centred colonies (*Strep. faecalis* or variants) predominated in both groups of birds. A representative number of these colonies were isolated and tested for their sensitivity to chlortetracycline (Table 6A). No detailed identification was made but the organisms were tested for gelatin liquefaction to distinguish *Strep. faecalis* from its variant *liquefaciens*. None of the highly resistant non-proteolytic strains of *Strep. faecalis* was found. It was apparent that a normal streptococcal flora had been re-established but there was now also a higher proportion of the more resistant type of *Strep. faecalis* var. *liquefaciens* in the control birds.

The birds were slaughtered 5 months after they had been given the last antibiotic supplement and the caecal population examined. The results which are included in Table 6B confirmed that the population in the previously antibiotic fed birds now resembled that of the controls and the more restraint strains of *Strep. faecalis* var. *liquefaciens* predominated in all the birds.

TABLE 6

Chlortetracycline resistance of faecal streptococci isolated from birds 3 months and 5 months after the last 400 p.p.m. chlortetracycline supplement.

Group	Organism	Nos. of strains	Growth on agar containing chlortetracycline p.p.m.			
			0	10	50	200
A. FAECES						
Control	Strep. faecalis	1	+	-	-	-
	var. liquefaciens	5	+	+	+	-
3 months after the last antibiotic supplement (faecal and caecal droppings)	Strep. faecalis	2	+	-	-	-
	Strep. faecalis	21	+	-	-	-
	var. liquefaciens	2	+	+	+	-
		1	+	+	+	±
B. CAECAL CONTENTS						
Control	Strep. faecium types	7	+	-	-	-
	Strep. faecalis var. liquefaciens	28	+	+	+	-
5 months after the last antibiotic supplement.	Strep. faecium	3	+	-	-	-
		1	+	+	+	-
	Strep. faecalis	1	+	-	-	-
	var. liquefaciens	24	+	+	+	-

The spread of antibiotic resistant strains in poultry plants

The presence of a highly resistant non-proteolytic strain of *Strep. faecalis* in birds fed on chlortetracycline supplements was confirmed in a recent survey of faecal streptococci in a poultry plant. This plant was eviscerating birds aged 10-12 weeks which had been fed until slaughter on rations containing 5 p.p.m. chlortetracycline. The faecal streptococci were counted on thallous acetate tetrazolium agar and the presence of the typical strongly reducing colonies of the resistant strain of *Strep. faecalis* was noted in many of the samples. Ten of these organisms isolated from the surfaces of 2 frozen eviscerated birds were tested for gelatin liquefaction and sensitivity to chlortetracycline. Only one out of the ten liquefied gelatin and this particular organism was very resistant as it could just grow on agar containing 200 p.p.m. chlortetracycline (c.f. 1 strain in Table 6). Of the 9 non-proteolytic strains, one was sensitive, 8 grew at 50 p.p.m. and 3 just grew at 200 p.p.m.

Discussion

The faecal streptococci most commonly found in birds reared on normal diets without antibiotic supplement are *Strep. faecalis* var. *liquefaciens* and *Strep. faecium*. Both these strains have been reported as common in poultry (Harrison & Hansen, 1950). Other references in the literature to the presence of *Strep. faecalis* may have in fact referred to *Strep. faecium* as reliable tests have been found for separating these two species only recently. Non proteolytic *Strep. faecalis* strains were seldom found in the poultry fed without antibiotic supplements in these investigations. Of 124 strains tested 118 liquefied gelatin so were classified as *Strep. faecalis* var. *liquefaciens*. Only 1 strain of *Strep. faecalis* var. *zymogenes* was isolated. A random collection of the isolates showed that there was a varying degree of sensitivity to the tetracycline compounds amongst the strains of *Strep. faecium* and *Strep. faecalis* var. *liquefaciens*, although none were resistant to 200 p.p.m. of chlortetracycline.

The addition of a chlortetracycline supplement to the rations had little effect on the total numbers of faecal streptococci in the faeces during the test period (5th to 12th week). Even when the 400 p.p.m. feed was given from the 6th to 8th week and again from the 10th to 12th week, the total numbers were virtually unchanged. However total viable counts on media containing chlortetracycline showed that a resistant population had developed in both the antibiotic fed groups of birds, and this result was confirmed when the caecal contents of the birds slaughtered at 12 weeks were analysed. This agrees with the findings of Sieburth *et al.* (1954) who demonstrated a chlortetracycline resistant population only one week after giving the birds a 10 p.p.m. supplement of chlortetracycline in the feed. Further evidence has been supplied by Smith and Crabb (1957) and Gordon and Tucker (1958) on changes in the resistance of the coliform population. The concentration of chlortetracycline in the caeca of the birds fed on the 25 p.p.m. supplement was only about 0.5 p.p.m. but the faecal streptococci which developed in the presence of the antibiotic were resistant to much greater concentrations than this.

Owing to the method of counting used for the faecal streptococci it was possible to demonstrate that although the total numbers were approximately the same in all three groups (Figure 2) there was a complete change in type as well as resistance. In the 12 control birds *Strep. faecium* strains were present at from 10^5-10^7/g. whilst the

Strep. faecalis var. *liquefaciens* types were less than 10^4/g. and almost all of these strains were inactivated by 10 p.p.m. chlortetracycline. In the 400 p.p.m. fed birds the situation was reversed, the *Strep. faecalis* strains outnumbered the *Strep. faecium* and related types by 100:1 and were resistant to 200 p.p.m. of antibiotic. These strains were no longer gelatin liquefiers and differed in other respects from the strains in the control birds. An intensive search was made to see if any similar strains could be found in the control birds. As shown in Table 4 four non-proteolytic strains (i.e. *Strep. faecalis*) were isolated but these were very sensitive to chlortetracycline. No characteristic strongly reducing faecal streptococci could be found in the poultry feed either. These organisms may have been present in such small numbers in the control birds that they could not be found or else they developed from the other *Strep. faecalis* strains through active multiplication in the presence of the antibiotic. When the antibiotic supplement was withdrawn, the streptococcal population reverted to normal the proteolytic variant of *Strep. faecalis* (*Strep. faecalis* var. *liquefaciens*) once more establishing itself, although there was a tendency for both the control population and that of the previously antibiotic fed birds to contain many more resistant types than had been found in the control birds at 12 weeks. A possible explanation of this would be that there was cross-contamination between the cages and Gordon & Tucker (1958) found a similar increase in resistance in the coliform population of the same control group of birds.

It is evident that the 10-12 week 'broiler' which has been given an antibiotic supplement until slaughter will contain a resistant faecal flora which may contaminate the eviscerated and processed carcases. Thus the use of the same antibiotic for extending the storage life of these carcases will only be really effective when the birds are stored at low temperatures where this resistant mesophilic population cannot multiply, spoilage then being due to the psychrophiles. There are of course quite different reasons for not using chlortetracycline for extending the storage life of eviscerated carcases without refrigeration from poultry reared without antibiotics [Shrimpton, 1957].

Some impressive claims have been made for the extension of the storage life of carcase meat held at warm temperatures, after the injection before slaughter or infusion at slaughter of a few p.p.m. oxytetracycline or chlortatracycline, (Deatherage, 1956; Ginsberg *et al.* 1957). It is interesting to infer, on the basis of the present results, that if the particular animals had been fed with the same antibiotic supplements up to the time of slaughter, it would probably not have been possible to inhibit the deep spoilage organisms, assuming that these had originated from the gut of the animal concerned. The behaviour of any resistant pathogenic oragnisms coming from the faecal population under these conditions is also one which requires careful consideration.

Summary

The two faecal streptococci (Lancefield group D) found most frequently in the gut of 10-12 week old birds were *Strep. faecalis* var. *liquefaciens* and *Strep. faecium*.

The inclusion of chlortetracycline in the feed, either at 25 p.p.m. throughout life, or at 400 p.p.m. for short periods, did not alter the total numbers of faecal streptococci present. It did, however, lead to the development in large numbers of a different,

very resistant, strongly reducing, non-proteolytic strain of *Strep. faecalis*, which replaced the other strains normally present.

Three months after discontinuing the chlortetracycline supplement, faecal samples indicated that the atypical strain had disappeared again from the birds previously given the chlortetracycline, *Strep. faecalis* var. *liquefaciens* once more becoming dominant. Caecal contents examined after 5 months confirmed this. At this time, however, there was also an increase in the proportion of resistant strains of *Strep. faecalis* var. *liquefaciens* in the control groups as compared with 12 week old birds.

The implications of these results in relation to the preservation of meat and poultry with the tetracycline compounds is that the use of an antibiotic in the feed may nullify its effect as a preservative.

Acknowledgments

The work described in this paper was carried out as part of the programme of the Food Investigation Organisation of the Department of Scientific and Industrial Research.

The author gratefully acknowledges the co-operation of Dr. R. F. Gordon of the Houghton Poultry Research Station. The experiments were made with the technical assistance of Messrs. J. Barlow and K. H. Robinson.

REFERENCES

Abbey, A., Darken, M.A., Kline, E. F., Kohler, A. R., Maturi, V. F., Miller, W. H. and Upham, S. D. (1956-7): Antibiotics Annual: New York, N.Y., Medical Encyclopedia Inc. p.831.

Barnes, E. M. (1956): *J. appl. Bact. 19*, 193.

Barnes, E. M., Ingram, M. and Ingram, G. C. (1956): *J. appl. Bact. 19*, 204.

Deatherage, F. E. (1956): Proc. 1st Int. Conf. Antibiotics in Agric., Nat. Acad. Sci., Nat. Res. Council, Washington, D.C.

Ginsberg, A., Hill, E. C. and Grieve, J. M. (1957): *Vet. Rec. 69*, 983.

Gordon, R. F. and Tucker, J. F. (1958): paper to 11th World's Poultry Congress, Mexico City. (To be published).

Grove, D. C. and Randall, W. A. (1955): Assay methods of antibiotics: New York, N.Y. Medical Encyclopedia Inc.

Harrison, A. P. and Hansen, P. A. (1950): *J. Bact. 59*, 197.

Shattock, P. M. F. (1949): *J. gen. Microbiol. 3*, 80.

Shrimpton, D. H. (1957): *J. Sci. Food Agric. 8*, 485.

Sieburth, J. McN., Jezeski, J. J., Hill, E. G. and Carpenter, L. E. (1954): *Poultry Sci. 33*, 753.

Smith, H. Williams and Crabb, W. E. (1957): *Vet. Rec. 69*, 24.

HEPATIC VENO-OCCLUSIVE DISEASE AND MEGALOCYTOSIS IN *SENECIO* POISONING OF HORSES

(Further investigations of "The Walking Disease" of Northwestern Nebraska)

by

KENNETH R. HILL, B.Sc., M.D., M.R.C.P.,

Professor of Pathology, The Royal Free Hospital School of Medicine, University of London.

and

H. M. MARTIN, V.M.D., A.M., Ph.D.,

Professor of Parasitology, School of Veterinary Medicine, University of Pennsylvania.

During the last seven years, the University College of the West Indies has made an intensive study of liver disease in that area. One of the several types of disease studied has proved to be veno-occlusive disease of the liver (Hill 1951, Hill *et al.* 1953, Bras and Hill 1956). This disease is not confined to humans in the West Indies but is found in animals and has a world-wide distribution (Hill and Bras 1957), and there is much evidence to show that it is caused by the pyrrolizidine group of alkaloids which are found in the family *Compositae,* (genus *Senecio*), as well as in the *Leguminosae* (genus *Crotalaria*) and *Boraginaceae* (genus *Heliotropum*) (Bras et al. 1957).

A perusal of the literature with the object of studying recorded cases of Senecio poisoning showed that poisoning by *Senecio Riddellii* T. & G. had been intensively studied by Hays and Van Es (1929), Van Es *et al.* (1929) and Carpenter (1938). Their studies had concerned a peculiar and fatal disease of livestock called the "Walking Disease" of Horses and Cattle in Northwestern Nebraska, which was experimentally reproduced by the feeding of the plant *Senecio Ridellii.* Photographs of the livers (gross) in the articles dealing with the "Walking Disease" in livestock resembled remarkably the appearance of the livers of fatal human cases of veno-occlusive disease (V.O.D.) in Jamaica (Fig. 1). As a consequence, the Department of Animal Pathology, University of Nabraska, was approached, and Dr. Skidmore, from that institution, was able to supply the authors with wet tissues and slides from the original cases dating back to 1929.

Gross and microscopic examination of these tissues showed that the pathology was that of hepatic veno-occlusive disease with, in some cases, the presence of hepatocellular megalocytosis (Bull 1955).

XUM

Material and Examination

The specimens were either slides stained H. and E., or tissues fixed in formalin. With the former, restaining was carried out in every case after a preliminary examination. The tissues were embedded in paraffin, and cut and stained by the following techniques: (i) H. and E., (ii) Picro-Mallory connective tissues, and (iii) Reticulin stain. Fat stains on frozen sections were also made.

Natural Field Cases

Horse No. (unknown):

History.—Diagnosis—" Necrobiosis et cirrhosis hepatitis equi senecio poisoning." (No other history).

Material.—Several portions of liver fixed in formalin.

Gross Examination.—The appearances were those resembling V.O.D. or chronic venous congestion (nutmeg liver) in humans (Fig. 1).

Microscopic Examination (A.23/57).—In the H. and E. stain (which showed the tissues had been poorly fixed), the liver looked grossly congested with huge lacunae of blood (Fig. 2) and occasional fibrinoid thrombi in the centrilobular veins. The pattern became clear, however, with special connective tissue stains, which showed blockage of the central veins, either by connective tissue (Fig. 3) or by recent fibrinous thrombi.

In addition, the usual cell column pattern within the lobule was lost, due to a generalised increase in size of the nucleus and cytoplasm of the cells similar to the " megalocytosis " described by Bull (1955) (Fig. 3).

Comment.—This was a case of acute-on-chronic V.O.D. with megalocytosis.

Horse No. 8797.

History.—This horse, a gelding aged 10 years, had been ailing for 22 days before dying on 7-6-26. It was emaciated, had a staggering gait, and had yellowish and injected conjunctivae. It had been grazing in an area in which were growing *Astragalus mollissimus* Tarr., *Oxytropis Lambertii* Pursh, *Lupines* and *Senecio spartoides* T. & G., and probably *Senecio Riddellii.*

Post Mortem.—Showed yellow, ascitic fluid in the abdominal cavity, with serous shreds on the liver which had a few subserous petechiae and a " nutmeg " pattern cut surface. There were also marked splenic subserosal haemorrhages.

Material.—Histological slides of liver and kidney.

Microscopic Examination, (A.17/57).—Liver: This showed marked fibrosis both centrilobular (due to V.O.D.) and portal. There were lacunae of blood suggestive of an acute exacerbation, and also some degree of megalocytosis (Figs. 2 and 3). The liver also had a small abscess containing organisms suggestive of actinomyces.

Comment.—Acute-on-chronic V.O.D. with megalocytosis.

XUM

Horse No. 8763.

History.—This animal, a sorrel mare aged 7 years, had been grazing in pasture for several months, and was destroyed by shooting on 1-6-26, within one to two weeks of onset of illness. This latter consisted of extreme depression and unease, profuse perspiration, nostrils dilated with roaring inhalation and a short, forced exhalation; head extended, constantly walking with inco-ordination of movement; conjunctivae markedly injected.

Post Mortem.—Liver showed shreds on diaphragmatic surface and a nutmeg cut surface; abdominal cavity, peritoneum showed a few serous shreds.

Material.—Histological slides of liver, heart and kidney.

Microscopic Examination, (*A.*19/56).—The liver showed fibrosis and V.O.D. with lacunae of blood and congestion (Figs. 2 and 3). The heart and kidney were not noticeably abnormal.

Comment.—The appearance suggested acute-on-chronic V.O.D.

Experimental Cases

Horse No. 53080.

History.—This was a bay horse which received 16 daily doses of dry Extract from *Senecio Ridellii* before dying on 9-1-32. The probable dosage was 10 grammes of Extract per day (4.56 grammes of dry Extract was prepared from 1 lb. of green plants).

Material.—Portions of liver, spleen and kidney, fixed in formalin.

Gross Examination.—The liver had an appearance similar to human V.O.D. Other organs not abnormal (Fig. 1).

Microscopic Examination, (*A.*22/57).—Liver shows extreme congestion and pooling of blood due to occlusion of the centrilobular veins, due to a thickening of the intima (Figs. 2 and 3). Reticulin stains show little damage to the lobular pattern except for the centrilobular occlusion which is partial or complete (Fig. 4), and which shows itself as knots of connective tissue. Spleen and kidney not abnormal.

Comment.—Acute V.O.D.

Horse No. 23721.

History.—This horse, a mare, was fully reported by Van Es *et al.* (1929) and was fed 172 lbs. of plants of *Senecio Riddellii* over 65 days. It lost weight substantially, and also appetite, and became apathetic. It was killed 46 days after the cessation of feeding the Senecio.

Post Mortem.—Showed ascites, the ascitic fluid as well as the conjunctivae mucosae and subcutis being tinged with yellow. The omentum and mesenteries were very oedematous. The kidneys showed advanced degeneration and the heart subepicardial ecchymosis. The liver had a mottled appearance and a bluish colour, and showed resistance to the knife and resembled the livers of animals dying from "the walking disease."

Material.—Portions of liver and kidney fixed in formalin.

XUM

Gross Examination.—Liver had an appearance similar to human V.O.D. (Fig. 1). The kidney was very pale and fatty, but not otherwise remarkable.

Microscopic Examination, (*A.*21/57).—H. and E. stained sections showed tremendously congested and poorly fixed tissues. Connective tissue stains, however, revealed occlusion of the central veins, sometimes partial and sometimes complete, to leave only an irregular area of fibrosis centrally (Fig. 2). Reticulin stains showed a fine intimal proliferation (Fig. 4), with increase of reticulin fibres radiating towards the periphery. The sections also showed an increase in portal fibrosis.

In addition there had been an acute exacerbation of the condition, in that there was intense congestion and pooling or lacunae of blood, with an occasional agonal fibrinous thrombus (Fig. 2).

The hepatic cells had a most pleomorphic appearance, rather grotesque, showing gross enlargement of both cytoplasm and nucleus. They resembled the megalocytosis of Bull (Fig. 3). The kidney showed tubular vacuolar degeneration.

Comment.—The liver showed chronic V.O.D. and megalocytosis.

Horse No. 53081 (?).

History.—This was a blazed face horse which received 16 daily doses of dry extract of *Senecio Riddellii* before dying on 10-1-32. The probable dosage was 10 grammes of extract per day (4.56 grammes dry extract was prepared from 1 lb. of green plants).

Material.—Four slides stained H. and E. from liver, spleen, kidney, heart.

Microscopic Examination.—The H. and E. slides showed poor fixation and intense congestion. However, on restaining with connective tissue stains, V.O.D. of the central vein, either complete or partial, with connective tissue, with pooling of blood in the sinusoids, was evident (Fig. 2). The kidney showed tubular vacuolation and the heart and spleen were not abnormal.

Comment.—Acute V.O.D.

Discussion

There is little doubt that the pathological changes in the liver of the disease of livestock known as "The Walking Disease" of horses and cattle in Northwestern Nebraska has been reproduced by the feeding of the plant *Senecio Riddellii* to horses by Hays and Van Es (1929) and Van Es *et al.* (1929).

It is of further interest that de Kock *et al.* (1931) had examined microscopic slides from the cases of Van Es *et al.* and that they considered the lesions to be similar to those of Dunsiekte or Enzootic Liver Disease of Equines in South Africa (which they believe to be caused by *Senecio* poisoning).

In 1928, Murnane and Ewart stated that Winton Disease of New Zealand, Dunsiekte of South Africa and Walkabout Disease of Northwestern Australia show similar liver "pathology" and de Kock *et al.* (1931) believe that the Walking Disease of Nebraska should be added to the list, to make a fourth disease having similar aetiology and liver "pathology".

XUM

PLATE 1

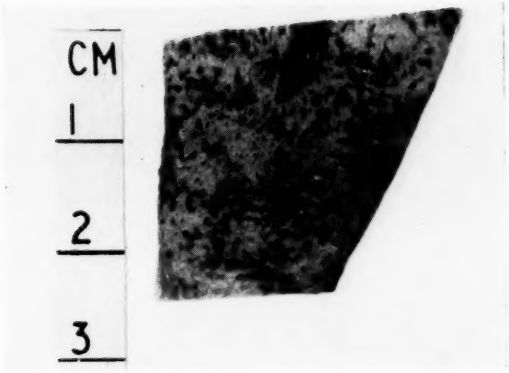


FIG. 1

Gross picture of liver specimen from horse fed with *Senecio Riddellii*, showing intense centrilobular congestion; this is similar to V.O.D. in the human.

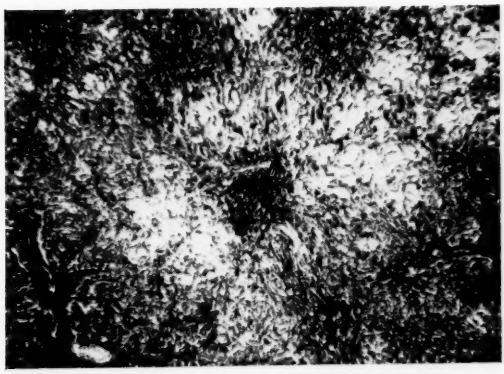


FIG. 2

Acute on chronic veno-occlusive disease.

This shows intense congestion with a knot of connective tissue centrilobularly. The open spaces or lacunae appear to be empty because of lysis of the blood within them, although close inspection shows that the blood in fact was present.
Picro-Mallory connective tissue stain x 20.

(Article by Hill and Martin on pages 345-350)

XUM

PLATE 2

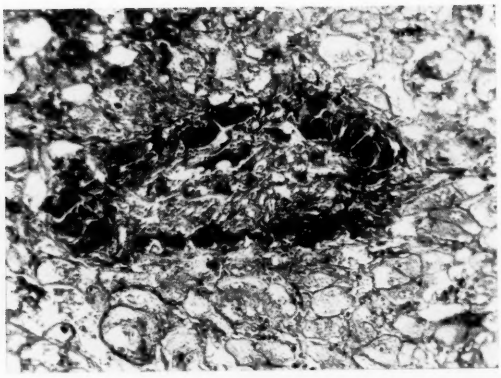


FIG. 3

Veno-occlusive disease and megalocytosis.

A characteristic occlusion of a centrilobular vein by intimal connective tissue hyperplasia. In addition the liver cells are pleomorphic and enlarged (megalocytosis). Picro-Mallory connective tissue stain x 125.

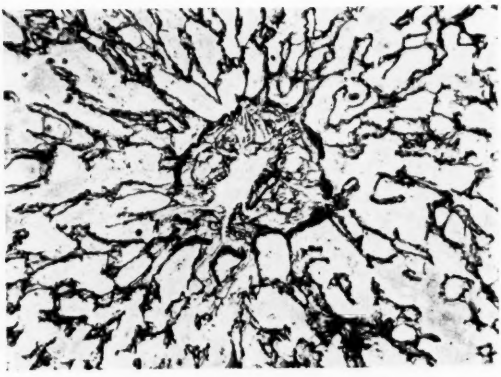


FIG. 4

Veno-occlusive disease.

Showing partial occlusion of a centrilobular vein by a fine intimal reticular framework.
Reticulin stain x 125.

(Article by Hill and Martin on pages 345-350)

XUM

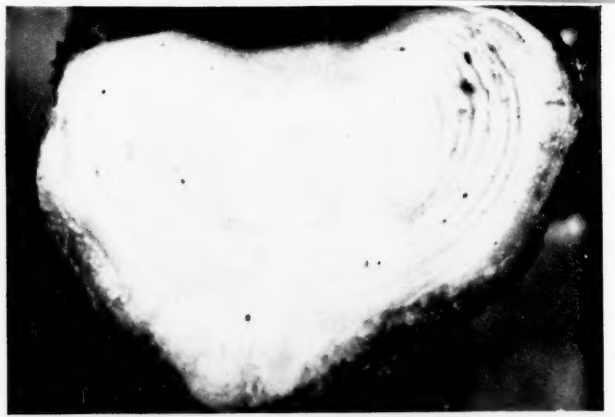


FIG. 1
Transverse section of 12/13 Thoracic disc. Note ventral protrusion of nucleus pulposus, and osteophyte.

FIG. 2
Protrusion of 4/5 Cervical disc. Note dura mater firmly adherent to the protruded mass.

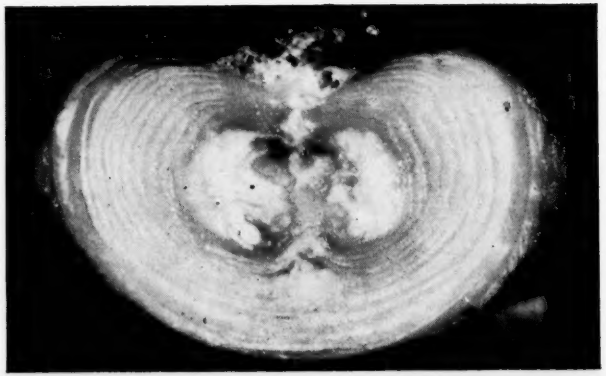


FIG. 3
Transverse section of 13 Thoracic/1st Lumbar disc. Note dorsal protrusion of nucleus pulposus.

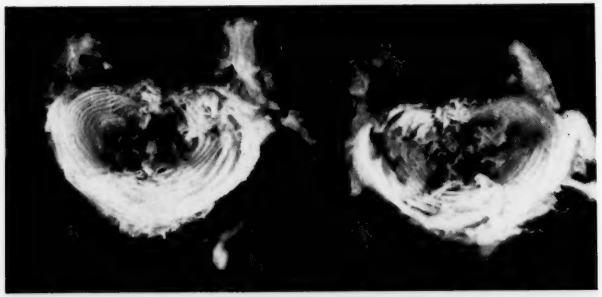


FIG. 4
Transverse section of 4/5 Lumbar disc. Note "' brown discolouration " and tattered appearance of the nucleus pulposus.

(Article by L. C. Vaughan on pages 350-355)

PLATE 2

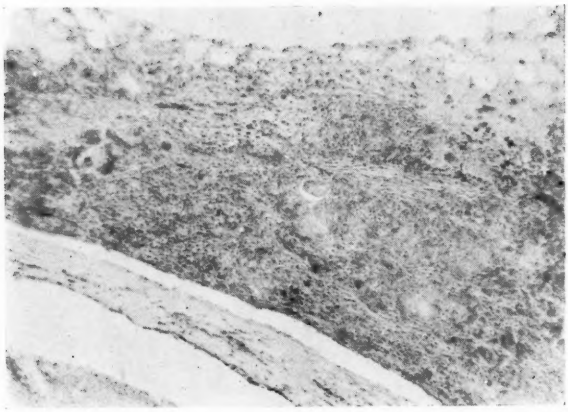


FIG. 5

Fibrous tissue enclosing foci of protruded disc material, external to the dura mater. Haematoxylin and Eosin. X90.

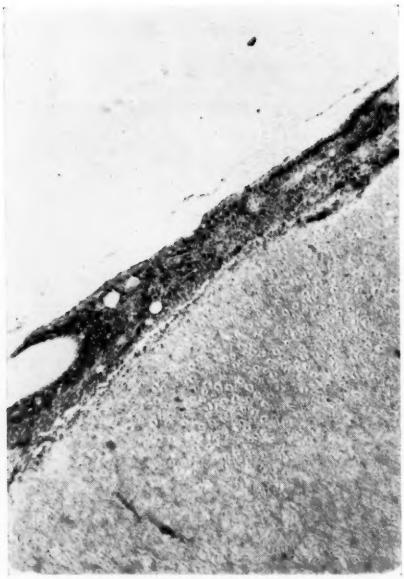


FIG. 6

Transverse section of cervical spinal cord. Note the leptomeningitis. Haematoxylin and Eosin. X90.

FIG. 7

Transverse section of cervical spinal cord. Note the cysts in the pia mater. Haematoxylin and Eosin. X90.

(Article by L. C. Vaughan on pages 350-355)

XUM

It is of further interest to note that on re-examination of the material of Van Es *et al.* (1929), the present authors have found two histological features, namely (a) veno-occlusive disease, and (b) megalocytosis, which had hitherto not been recognised.

Occlusion of the hepatic veins of the liver has been found in humans and cattle in the West Indies (Bras and Hill 1956; Bras *et al.* 1957), and also in various other parts of the world (Hill and Bras 1956), e.g., India (humans), South Africa (humans, horses and cattle), Australia (sheep, horses and cattle). The aetiological agent has been in some cases suspected to be, and in others proved to be, one of the pyrrolizidine (*Senecio*) group of alkaloids.

The pathogenesis of the lesion is not yet understood and intensive research into this aspect of the disease is at present taking place in the laboratories of Professor Bras at the University College of the West Indies, and in the laboratories of the Royal Free Hospital.

The pathology is venous occlusion, with consequent damming back of the blood to produce intense sinusoidal congestion and lacunae formation. Such histological pictures have been described by most authors, although the actual vascular lesions have not been identified. For example, Theiler (1918 a and b) describes centrilobular stasis and lagoons of blood in his description of Dunsiekte, and also after feeding experiments with *Senecio* and *Crotalaria*, using horses and cattle, and his microphotographs support this. De Kock *et al.* (1931) give descriptions of lesions which are similar to those found in known veno-occlusive disease, and more recently Bull (1957) has reproduced a picture of classical veno-occlusion in his article on *Crotalaria* poisoning.

In the re-examination of the Nebraska material, the authors have found a condition described by Bull as "megalocytosis". This is an enlargement of the liver cells per se, and is not due necessarily to regeneration of liver cells, such as one finds in cirrhosis. Mention of enlargement of liver cells was made by Theiler (1918 a) and the subject is currently under study by Bull (1955), and by one of us (K.R.H.). At the moment is it suggested that the appearance may be due to a hepatotoxic stimulation of the liver cells which may ultimately result in a primary hepatic neoplasm (Schoental *et al.* 1954 and 1955).

Summary

1. In 1929, Van Es *et al.* reproduced "the Walking Disease" of Horses and Cattle in Northwestern Nebraska by feeding horses with *Senecio Riddellii*.
2. A re-examination of specimens of the livers from Nebraska experiments of 1929 has revealed the presence of (a) veno-occlusive disease, and (b) megalocytosis of the liver.
3. The disease appears to be similar to other diseases in humans, cattle, horses and sheep which have a world-wide distribution, and are probably caused by the ingestion of the pyrrolizidine group of alkaloids (*Senecio*).

We wish to acknowledge the help given by Dr. L. V. Skidmore, D.V.M., of the Department of Animal Pathology and Hygiene, College of Agriculture, the University of Nebraska, Agricultural Experiment Station, Lincoln 3, Nebraska, in supplying information, material and encouragement in this investigation.

XUM

BIBLIOGRAPHY

Bras, G. Berry, D. M., Gyorgy, P. (1957): *Lancet, 1,* 960.
Bras, G., and Hill, K. R. (1956): *Lancet, 2,* 161.
Bull, L. B. (1955): *Aust. vet. J.,* Feb., 33.
Bull, L. B. (1957): *Aust. vet. J.,* March, 49.
Carpenter, P. T. (1938): Vet. Bull. Supplmt. to the Army Med. Bull., *32,* 33.
de Kock, G., de Toit, P. J., and Stey, D. J. (1931): 17th Rep. of Director of Vet. Services and Animal Industry, Union of S. Africa, 617.
Hays, C. H., and Van Es, L. (1929): (Monograph) State of Nebraska, Dept. of Agriculture, Lincoln.
Hill, K. R. (1951): Trans. Tenth Conference on Liver Injury, Jos. Macy, Jr. Fdtn., New York.
Hill, K. R., and Bras, G. (1957): Geographic Pathology of Hepatitis in Hepatitis Frontiers, Little, Brown & Coy., Boston.
Hill, K. R., Rhodes, K., Stafford, J. C., and Aub, R. (1953): *Brit. med. J., 1,* 117.
Murnane, D., and Ewart, A. J. (1928): Bulletin No. 38, Council for Scientific and Industrial Research, Commonwealth of Australia.
Schoental, R., Head, M. A., and Peacock, P. R. (1954): *Brit. J. Cancer, 8,* 458.
Schoental, R., and Head, M. A. (1955): *Brit. J. Cancer, 9,* 229.
Theiler, A. (1918a): 5th and 6th Reps. of Director of Vet. Services, South Africa, 9.
Theiler, A. (1918b): 7th and 8th Reps. of Director of Vet. Services, South Africa, 105.
Van Es, L., Cantwell, L. R., Martin, H. M., and Kramer, J. (1929): College of Agriculture, Univ. of Nebraska, Agricultural Experiment Station Research Bulletin 43. Lincoln, Nebraska.

STUDIES ON INTERVERTEBRAL DISC PROTRUSION IN THE DOG

by

L. C. VAUGHAN, M.R.C.V.S.

Department of Surgery, Royal Veterinary College, Streatley, Berks.

3. PATHOLOGICAL FEATURES

The aetiology, pathogenesis, and diagnosis of intervertebral disc protrusion in the dog have been discussed in previous papers. (Vaughan 1958a and 1958b.) Observations on the pathology of the disease obtained from the autopsy examination of 32 dogs with disc protrusion are presented.

Materials and Methods

Thirty-two dogs which were destroyed at the request of their owners or died from other causes were examined *post mortem.* Of the 22 which were destroyed 3 were quadriplegic at the time. One dog had suffered from paraplegia for two months prior to the development of a cervical protrusion. Paraplegia was the reason for the destruction of 16 dogs and the length of time between death and the onset of symptoms varied from 1 to 455 days. Ataxia, partial paralegia and cervical pain were the reasons for the destruction of the remaining 3 dogs. The 10 dogs which died included 3 which were primarily paraplegic but later developed an ascending paralysis and became moribund. The hazards of myelography and anaesthesia undoubtedly contributed to the death of 4 dogs. One of these was quadriplegic and three paraplegic. Surgical intervention could possibly be held indirectly responsible on 3 occasions, although in one instance in which the dog died 7 days after operation, death could have been ascribed to severe damage to the spinal cord by the protrusion.

XUM

The carcase was examined to eliminate the possibility of intercurrent disease. The spine from atlas to coccyx was radiographed. The radiographical definition of the lesions was better than that obtained when the spine was radiographed *in situ*. This technique when correlated with the pathological findings, was of great benefit in the interpretation of radiographs of subsequent patients. Finally, the contents of the neural canal were examined after removal of the dorsal spines and laminae.

Gross Findings

Types of Protrusion

It has been observed that the nucleus pulposus may be extruded in any one of three directions giving rise to dorsal, ventral or intraspongious protrusions. The two latter are of minor significance.

Ventral protrusion. The nucleus is displaced through the ventral annulus and, according to Debard (1949) and Hansen (1952), osteophytes, characteristic of spondylosis deformans, form on the ventral aspect of the adjacent vertebrae. One case only of ventral protrusion was seen (Fig. 1) and it was accompanied by the presence of osteophytes. It cannot be said, however, that osteophytes are the prerogative of ventral protrusion since they have been found in 8 dogs with dorsal protrusions.

Intraspongious protrusion. The nucleus pulposus is projected into the cancellous bone of the adjacent vertebra. This lesion is common in man and is termed Schmorl's Node. Hansen (1952) records having seen this type in the dog. It was not seen in this series of observations either radiographically, macroscopically on post-mortem inspection or at histological examination.

Dorsal protrusion. The nucleus pulposus is eccentrically placed in the disc and hence the annulus fibrosus is thinner dorsally than ventrally. Consequently it is not surprising that almost all protrusions of the nucleus occur dorsally. It is this type of lesion which is responsible almost entirely, on account of its interference with the spinal cord, for locomotory disease. Dorsal protrusions were found in all the spines examined *post mortem*.

Two forms of dorsal protrusion were recognised macroscopically. In one the annulus and the dorsal common spinal ligament had ruptured, permitting the extrusion of nucleus into the spinal canal (Type 1, Hansen 1952). The protrusion, which as a rule was large, produced epidural haemorrhage and invoked an inflammatory reaction in its near vicinity. The spinal cord therefore, was liable to become injured and this was found to be the case in 30 spines. The other form consisted of a bulging of the annulus and dorsal spinal ligament but since these structures remained intact the nucleus could not escape (Type 2, Hansen 1952). Generally this type of protrusion was small, caused no haemorrhage and appeared to interfere but little with the spinal cord. Nevertheless, in two dogs which had been partially paraplegic only this form of protrusion was demonstrated. It was not unusual to find types 1 and 2 coexisting in the same spine.

Distribution of Lesions

In the 32 spines, 67 dorsal protrusions were discovered. Of these 7 were cervical and 60 were thoracolumbar (Table No. 1). Single protrusions were present in 18

XUM

spines (1 cervical, 17 thoracolumbar). Multiple protrusions were found in 14 spines which contained 49 protrusions (6 cervical, 43 thoracolumbar), giving an average of 3.5 per spine. The discs most commonly affected were those in the region of the thoraco-lumbar junction. Indeed, protrusions of the 13Th./1 L. and 1/2 L. discs together accounted for 31% of the total. The protrusion of the 9/10th. disc was the only one found at the discs to which the conjugal ligament lends support, namely those between the first and tenth thoracic vertebrae.

TABLE 1

Distribution of 67 dorsal protrusions present in 32 dogs at autopsy.

Disc	*Number of protrusions*	*Disc*	*Number of protrusions*
C. 2/3	3	Th. 12/13	7
C. 4/5	2	Th. 13/L. 1	11
C. 6/7	2	L. 1/2	10
C. 7/Th. 1	2	L. 2/3	5
Th. 9/10	1	L. 3/4	9
Th. 10/11	2	L. 4/5	6
Th. 11/12	6	L. 5/6	1

Macroscopic Appearances of Dorsal Protrusions

Type 1. Rupture of the common dorsal spinal ligament was dorso-medial in 36, right or left dorso-lateral in 3, making a total of 39 protrusions. Sometimes the protruded material had spread like the head of a mushroom to cover part of or the entire dorsal surface of the disc. At other times it was small and restricted to the site of rupture, its size being dependent on the amount of nucleus extruded. In shape the protrusions were flat or raised, circular, oval, conical or irregular. As a rule the material was of a firm consistency, often it was calcareous and if of long standing dense. Occasionally in the early case it was pultaceous. In the majority of instances the protrusion was adherent to the dura mater and quite often it was impossible to remove the cord without detaching particles of the protruded mass (Fig. 2). Recent protrusions were blood stained but older cases were white or grey in colour. Epidural haemorrhage varied in degree and extent, being most marked in the recent case. Haemorrhage, which involved dura and epidural fat, was restricted to the near vicinity of the protrusion. Large protrusions which, from the symptoms and history were known to have been present for sometime, produced permanent indentation of the ventral aspect of the cord.

Associated with recent protrusions, material identical in composition with that at the site of rupture has been found estranged from the parent tissue either anteriorly or posteriorly and adherent to the sides or floor of the vertebral canal.

It was necessary to remove the protruded nuclear material in order to discover the point of rupture in the dorsal spinal ligament but the track through the annulus could be demonstrated by transverse section only (Fig. 3). It was not easy to locate the track always since it was not straight in all instances and could be missed if section were made in one plane only. In this connection sagittal sections of the region, cut according to the Gough-Wentworth (1947) technique, which was originally used for sections of lung, have been useful (Vaughan, 1958a; Figs. 6 and 7). The remaining nucleus pulposus was reduced in size commensurate with the amount extruded. It was usually yellowish white, semi-solid and sometimes calcified. On one occasion the central part of a disc was brown in colour (Fig. 4). This pigmentation

has been observed frequently in man and is termed "brown degeneration", Schmorl (1929). It is interesting to note that in the dog Hansen (1952) found "brown degeneration" in a third of the discs in this type of protrusion, and considered that the pigment was haemorrhagic in origin.

Type 2. This lesion was constantly dorso-medial or dorso-lateral in position but it varied greatly in appearance. It was seen as a transverse bulge of the whole dorsal aspect of the disc; a longitudinal midline elevation with its summit over the disc; or it consisted of bud-like prominences on one or both sides of the midline. The protrusion was firm, smooth and rarely adhered to the dura mater. There was never any evidence of epidural haemorrhage. Transverse section of the disc showed the annulus and dorsal spinal ligament to be intact but it bulged dorsally. Occasionally there was rupture of some inner fibres of the annulus but in not one of the 28 examined was the annulus found to be completely ruptured permitting the nucleus to bulge the dorsal ligament, as described by Hoerlein (1953). It was unusual to find any macroscopic change in the nucleus but sometimes it had become yellowish white and semi-solid, or even calcified.

Macroscopic Appearances of the Spinal Cord and Meninges

The macroscopic changes were largely dependent on the duration and size of the protrusion and were restricted to its vicinity. The recent case was associated almost invariably with sufficient haemorrhage to cause localised staining of the whole circumference of the dura. This haemorrhage is probably primarily responsible for the adhesion which develops between the extruded nucleus and the dura mater in the type 1 protrusions. When the protrusion was large and of some standing it produced a permanent indentation on the ventral aspect of the cord. Multiple protrusions affecting neighbouring discs caused a characteristic corrugation of the cord. Myelomalacia of the spinal cord at the compression sites was not an uncommon finding and cord matrix escaped readily if the dura were incised.

Histology

Spinal Cord and Meninges

The spinal cords from 8 of the dogs were examined. In all of these cases sections were taken from the cervical, thoracic, anterior and posterior lumbar regions, and stained with haematoxylin and eosin. Staining for myelin degeneration was limited to Weil's method (1928).

Spinal cord. The histological structure of the cord at the site of protrusion had been destroyed beyond recognition in four dogs. In two no abnormality was detected in the cord anterior or posterior to the lesion but in the third there was a distortion of cord tissue posteriorly. The fourth animal differed from the others in that it died as a result of an ascending paralysis 7 days after protrusion of a thoraco-lumbar disc. Besides the destruction of cell structure at the protrusion there was a similar damaged region in the cervical cord, and also a generalised gliosis and myelin degeneration in the posterior lumbar cord.

The neuropathic lesions that were observed in the other cases mostly affected the cord and/or nerve roots in the near vicinity of the protrusion. Gliosis of grey and white matter and varying degrees of demyelination were common findings.

XUM

The basic effect of cord compression is the degeneration of nerve fibres and the severity of degeneration depends to some extent on the duration and degree of compression. At the same time the ventral spinal artery may be obliterated and as a consequence the nutrition of that segment of cord is removed. Generally speaking these two factors explain why different pathological lesions are found. What is not understood is the individual variation by way of symptoms, and hence the pathological lesions, that occur in response to protrusions of similar size and nature at any one disc e.g. one dog becomes paralysed while the other is merely incoordinate.

Meninges. In four cases the dura was thickened where it had been in contact with the protrusion. There was also evidence on its external surface of inflammatory tissue enclosing groups of cells which resembled those of the nucleus pulposus (Fig. 5).

A leptomeningitis, which chiefly involved the cervical and thoracic regions of the cord but which varied in degree, was observed in six dogs (Fig. 6). In each case myelography, using Lipiodol, had been performed some time previously. Whether the Lipiodol, which has irritant properties, can be incriminated as the cause of this inflammation of the pia remains a matter for conjecture. In this connection it is interesting to note that one of the two cases in which a meningitis did not develop was destroyed the day after myelography and presumably before the pia could have been greatly influenced by the oil.

An extraordinary histological lesion of the pia mater was observed in the cervical cord from a Dachshund which had been paraplegic for 455 days. Myelography had been carried out 5 days after the onset of symptoms. The pia appeared to have given rise to multiple cysts each apparently lined with pseudo-epithelium (Fig. 7). They occurred in the whole circumference of the cord which in consequence was compressed. In the vicinity of the cysts there was myelin degeneration. It may well be argued that the Lipiodol was to blame for the initiation of this unusual lesion. While this may be so it must be stated that untoward sequelae have not so far been seen following its use, even after longer periods, in those dogs which recovered from the effects of disc protrusion.

Protruded Disc Material

Sections of this material were stained with haematoxylin and eosin. It consisted for the most part of cells identical to those of the nucleus pulposus. In recent cases large numbers of red blood cells were found, chiefly at the periphery but also dispersed throughout the material. Fibrous tissue occurred at the edge of the protrusion as well as between small areas of nuclear cells. Sometimes collagen fibres, which probably had become detached from the dorsal spinal ligament, were demonstrated.

Discussion

The foregoing observations support the view that the commonest route of displacement of the nucleus pulposus of the intervertebral disc of the dog is in a dorsal direction. It has been established also that dorsal protrusions can be divided into two categories depending on whether or not the nuclear material enters the spinal canal. In the so-called " type 1 " protrusion a rupture occurs in the dorsal annulus fibrosus and spinal ligament, and nucleus pulposus substance herniates into the neural canal. As there is little space between the spinal cord and the walls of the spine the former structure must be endangered. The degree of damage to the cord is proportional

to the size of the protrusion and the amount of haemorrhage and inflammation that has been produced. It seems likely that the signs and symptoms exhibited by the animal are largely dependent on these factors. In this investigation type 1 protrusions were demonstrated in all but two of the 32 dogs which were affected with disc protrusion subjected to examination *post mortem*. Protrusions of the "type 11" variety are characterised by a bulging of the dorsal aspect of the disc into the spinal canal. There is a notable absence of haemorrhage and the cord is but mildly compressed. This was the only lesion found in the remaining two dogs, which incidentally had been only partially paralysed. In summary it may be said that the major symptom-producing lesion is the "type 1" protrusion.

Despite the fact that all the discs degenerate at the same rate it is found that the discs most prone to protrusion are those at or near the thoraco-lumbar junction and in the neck. There is little doubt that this particular distribution of protrusions is closely associated with trauma since it is these regions which suffer the major stresses and strains of the dog's everyday activities.

The spinal cords of several dogs which had not recovered limb function following a disc protrusion were subjected to histological examination. As a rule the most severe neuropathic lesions were found at and closely adjacent to the site of the protrusion. A more extensive destruction of cord structure, however, was found in those dogs which apparently had died as a result of an ascending paralysis. The pia mater, particularly in the cervical and thoracic regions, was seen to be inflamed in each of six dogs that had been subjected previously to a myelographic examination. Probably some degree of leptomeningitis is always produced when an irritant contrast medium (in these cases, Lipiodol) is used but the author has not seen any untoward clinical sequelae in dogs subjected to the procedure and which recovered the use of their limbs subsequently.

The constituent parts of the protruded material have been demonstrated histologically. The mass is largely made up of cells so closely resembling the cells of the nucleus pulposus as to leave no doubt of their origin. That the protrusion evokes an inflammatory reaction from the local tissues is evidenced by the presence of fibrous tissue surrounding the whole mass and also interspersed between areas of these nucleus pulposus—like cells.

Acknowledgments

Grateful thanks are extended to I. H. Pattison, B.Sc., M.R.C.V.S., Agricultural Research Station, Compton, for the histological examination of the spinal cords and to Dr. E. E. Payne, Royal Infirmary, Cardiff, for introducing the author to the adapted Gough-Wentworth technique of spine section.

BIBLIOGRAPHY

Debard, H. (1949): "Contribution à l'anatomie pathologique du disque intervertebral chez les carnivores". Thesis. Alfort, Paris.
Gough, J. & Wentworth, J. E. (1947): Proc. 9th Int. Cong. on Indust. Med. London.
Hansen, H. J. (1952): *Acta orthop. scand.*, Suppl. 11.
Hoerlein, B. F. (1953): *Amer J. vet. Res.*, *14*, 260.
Schmorl, G. (1929): *Klin. Wschr.*, *8*, 1243.
Vaughan, L. C. (1958a): In press.
Vaughan, L. C. (1958a): *Brit. vet. J.*, *114*, 105.
——————— (1958b): *Ibid.*, *114*, 203.
Vaughan, L. C. (1958b): In press.
Weil, A. (1928): *Arch. Neurol. Psychiat.*, 20, 392.

XUM

SOME CHARACTERISTICS OF TYPE A STRAINS OF PASTEURELLA MULTOCIDA

By G. R. CARTER, D.V.M., M.S., D.V.Sc.

Department of Research, Ontario Veterinary College, Canada.

The main purpose of this note is to draw attention to several differential characteristics of type A strains of *Pasteurella multocida.* It is also hoped to resolve several inconsistencies that have arisen in the interpretation of phenomena observed in type A cultures.

Four types of *P. multocida* have been recognized on the basis of serological differences in capsular substance (Carter, 1955). Bain (1945) has studied the lipopolysaccharide of type B (Robert's (1947) type 1) strains in detail. These strains are now considered to be the cause of epizootic pasteurellosis or haemorrhagic septicaemia of cattle (Bain, 1957).

Type A strains can be distinguished from the other varieties of *P. multocida* by the presence in their capsular substance of hyaluronic acid (Carter and Annau, 1953). Freshly isolated cultures may possess sufficient hyaluronic acid to produce a colonial growth that distinguishes them colonially from types B, C, and D. Webster and Burn (1926), who first drew attention to these cultures, described them as moist, viscid and slightly spreading. The colonies resemble closely those of freshly isolated strains of *Klebsiella pneumoniae.*

There has been considerable confusion as to the place of these mucoid cultures in the classification of *P. multocida.* Webster and Burn dealt with these cultures as though they were another colonial variant distinct from the D and G variants described by DeKruif (1921). This approach was followed by the author in his early work but was abandoned when it was determined that from a serological standpoint these mucoid strains comprised one variety, viz., type A.

The status of the mucoid cultures was also confused by difficulties in performing precipitation tests with extracts containing capsular substance. A failure to carry out satisfactory precipitation tests with the mucoid cultures was thought to be due to the non-antigenicity of the capsular substance because it was composed of predominantly hyaluronic acid (Carter and Annau, 1953). However, the haemagglutination test (Carter, 1955) has shown that the capsular substance of type A strains is serologically reactive. Considerable difficulty has been experienced in obtaining immune sera giving satisfactory precipitin and haemagglutinating titres.

After frequent transfer on artificial media the colonies of type A strains tend to lose their mucoid character. Many type A laboratory strains have lost most if not all of their capsular substance. These cultures yield smooth colonies rather than the mucoid colonies seen in many fresh isolates.

Several hundred strains of *P. multocida* have been identified as type A by the indirect haemagglutination test employing the capsular substance to treat the red blood cells. A great variation has been noted in different type A cultures in regard to colonial appearance and virulence for mice. The " mucoidness " of strains may vary

from cultures that produce colonies that are discrete and only slightly mucoid to cultures that produce a markedly moist, viscid, confluent growth. Virulence for mice varies from those highly pathogenic strains that kill in 48 hours in dilutions of broth as high as 10^{-9} (0.5 ml. dose) to those strains that may take a week or more to kill in a lower dilution (Carter and Bigland, 1953). These observations and others indicate that the type A or group A category contains strains which may differ from one another considerably. They do, however, have in common an identical capsular component.

Some type A strains resemble *Klebsiella pneumoniae* in that full grown cultures on solid media undergo decapsulation. This decapsulation is so marked that a culture that has yielded generous growth will undergo a remarkable flattening within 18 hours after removal from the incubator (Carter and Bigland, 1953). That it is principally a matter of decapsulation is indicated by the loss of the large capsule as demonstrated by Jasmin's capsule stain (Jasmin, 1945) or the alcian blue technique (Gurr, 1956). Adams and Park (1956) have found that in *Klebsiella pneumoniae* an autolytic enzyme is released from phage infected cells.

The capsulated type A strains can be recognized by the action of staphylococcal hyaluronidase on the production of capsular substance. This effect can be demonstrated readily if a culture of a beta-hemolytic *Micrococcus aureus* is inoculated transversely over the streak lines of the pasturella. After overnight incubation the colonies of pasteurella in proximity to the micrococcus are greatly reduced in size. This reduction in size results from the loss of the large hyaluronic acid-containing capsule due to the action of the bacterial hyaluronidase. This same phenomenon has been described by Murray and Pearce (1949) in strains of mucoid streptococci.

Some of the chemical properties of a hyaluronic acid preparation recovered from what was believed to be a type B strain of *P. multocida* were described previously (Carter and Annau, 1953). A subsequent study of several hundred cultures of *P. multocida* has shown, as Bain (1954) pointed out, that cultures possessing capsules composed of a large proportion of hyaluronic acid are not type B.

It is now known that type A strains of *P. multocida* are the most frequent cause of fowl cholera (Carter, 1955). Studies on the active immunization of fowl should take into consideration the role of this capsular substance. At present there is a lack of information on the properties of the capsular substance of the different types of *P. multocida.*

REFERENCES

Adams, M. H. and Park, B. H. (1956): *Virology* 2, 719.
Bain, R. V. S. (1954): *Brit. vet. J.*, 110, 492.
Bain, R. V. S. (1957): *Ceylon vet. J.*, 5, 1.
Carter, G. R. and Annau, E. (1953): *Amer. J. vet. Res.*, 14, 475.
Carter, G. R. and Bigland, D. H. (1953): *Can. J. comp. Med.*, 17, 473.
Carter, G. R. (1955): *Amer. J. vet. Res.*, 16, 481.
Carter, G. R. (1957): *Amer. J. vet. Res.*, 18, 437.
DeKruif, P. H. (1921): *J. exp. Med.*, 33, 773.
Gurr, E. (1956): A Practical Manual of Medical and Biological Staining Techniques, Interscience Pub., New York.
Jasmin, A. M. (1945): *J. Bact.*, 50, 361.
Murray, R. G. E. and Pearce, R. H. (1949): *Can. J. Res.* E, 27, 254.
Roberts, R. S. (1947): *J. comp. Path.*, 57, 261.
Webster, L. T. and Burn, C. G. (1926): *J. exp. Med.*, 44, 343.

XUM

TUBERCULOSIS IN A WALLABY CAUSED BY THE AVIAN TYPE TUBERCLE BACILLUS

by

I. W. LESSLIE, B.V.Sc., M.R.C.V.S.

Central Veterinary Laboratory, Weybridge.

Seventeen cases of tuberculosis in marsupials in which the type of the infecting organism has been studied, have been reported in the literature. In 10 of these, the disease was caused by tubercle bacilli of the bovine type, in 2 by human type, and in the other 5 by avian type tubercle bacilli. Lucas (1925) was the first to investigate tuberculosis in a marsupial. The disease occurred in a rufous rat-kangaroo (*Aepyprymnus rufescens*) which died 6 weeks after arrival in the London Zoological Gardens, and the organism isolated was of the avian type. Two cases of tuberculosis in marsupials were reported by Griffith (1928); a captive rufous rat-kangaroo with extensive pulmonary lesions was found to be infected with the bovine type of tubercle bacillus, and a Kangaroo Island kangaroo (*Macropus fuliginosus*) died in the London Zoo with generalized tuberculosis caused by the avian type. Stableforth (1929) described tuberculosis in the abdominal organs of a wallaby which had been kept in a small paddock together with fowls, rabbits and other small rodents. Cultural tests and animal inoculations showed that the organism was of the avian type. Hamerton (1932) reported the death in the London Zoological Gardens of a black-faced kangaroo (*Macropus giganteus melanops*) infected with avian type tubercle bacilli in the pancreas. Stünzi (1950) described tuberculosis in the lungs of 2 kangaroos caused by the human type. Teuscher (1957) reported 14 cases of tuberculosis in kangaroos in the Zoological Gardens in Zurich. Typing tests carried out on the organisms isolated from 9 of these demonstrated tubercle bacilli of the bovine type. A tuberculous abscess in the spinal cord of a wallaby (*Macropus bennetti*) was found by Tilden & Williamson (1957) to be due to avian tubercle bacilli.

A further case of tuberculosis in a marsupial has been investigated recently and the organism isolated has been shown to be *Mycobacterium tuberculosis*, avian type.

History and Post-mortem Findings

The subject was a large wallaby (*Macropus rufogrisens* or *bennetti*), of unknown age, which was born in Whipsnade Zoological Park and whose forebears came to Whipsnade approximately 20 years ago. This animal, together with more than 100 other kangaroos and wallabies, was on free range in the Park. The animal was found dead and the general condition of the carcase was poor. Post-mortem examination revealed yellow caseous, nodules up to 2 mm. in diameter throughout the liver and spleen. No other lesions were noted other than some minor pneumonic congestion.

Portions of the liver and spleen were submitted for laboratory examination.

Laboratory Tests

Very numerous acid-fast bacilli, mostly short but some filamentous forms, were seen in films of the lesions from both liver and spleen.

XUM

Material from the lesions was pooled and after treatment with oxalic acid was inoculated intramuscularly into the right thighs of 2 guinea-pigs. Three weeks after inoculation, single intradermal comparative tuberculin tests were carried out on the guinea-pigs and both animals gave specific avian reactions. The guinea-pigs were examined at necropsy 6 weeks after inoculation. The lymph nodes local to the site of inoculation were enlarged and caseous and acid-fast bacilli were seen in films of these lesions. There were no apparent lesions elsewhere.

The treated pooled material from the wallaby lesions was also inoculated on to Dorset's egg, Lowenstein-Jensen and Stonebrink media. Colonies of acid-fast bacilli were visible in all tubes in 14 days. Growth from 21 days onwards was profuse with over 100 colonies growing in every tube, and was characteristic of the avian type tubercle bacillus.

The virulence of the strain recovered on primary culture was tested by further animal inoculations. Two guinea-pigs were inoculated intramuscularly, one with 1 mg. and the other with 0.1 mg. of moist culture, and 2 rabbits and 2 chickens were inoculated intravenously, one of each with 1 mg. and one with 0.01 mg. Tuberculin tests were carried out on the guinea-pigs 4 weeks after inoculation and both showed specific avian sensitivity. The guinea-pigs were killed 6 weeks after inoculation and necropsy revealed caseous lesions in the inguinal and lumbar lymph nodes of both animals; no other lesions were found. The rabbit inoculated with 1 mg. of culture died with the Yersin or septicaemic type of tuberculous infection in 13 days; no focal lesions were discernible macroscopically but the liver was enlarged and pale throughout and the spleen slightly enlarged and mottled. Very numerous acid-fast bacilli were present in the liver and spleen and smaller numbers were seen in films of the kidneys. The rabbit inoculated with 0.01 mg. of culture died 41 days after inoculation and the chicken inoculated with 1 mg. died at 73 days; both revealed generalized lesions of tuberculosis at autopsy. The remaining chicken, inoculated with 0.01 mg. of culture, was killed at 12 weeks and had numerous lesions of tuberculosis in the liver, spleen and kidneys. Avian type growth was also obtained on culture media from lesions found in the guinea-pigs, rabbits and chickens.

From the results of these biological and cultural tests the organism was classified as *M. tuberculosis,* avian type.

Material was selected for histological examination from the liver and spleen of the wallaby. Some areas of necrosis were present in the lesions which were composed mostly of epithelioid cells, together with some lymphocytes, polymorphonuclear leucocytes and giant cells, and large numbers of acid-fast bacilli. Streaks of fibrous tissue were seen around the periphery of some lesions.

Discussion

All wallabies at Whipsnade have the freedom of the Park and are able to come into contact with almost all of the 2,000 odd mammals and birds kept there. Many birds, including jungle fowl, pea fowl, guinea fowl, ducks and geese, are full-winged and have the freedom of the whole Park.

These cases of tuberculosis are of special interest because of the low position occupied by marsupials on the mammalian scale. Members of this order are susceptible to casual infection with avian, bovine and human types of tubercle bacilli.

XUM

All the cases reported have been of animals in captivity and there have been no reports in the literature of tuberculosis in marsupials in the wild state, but autopsy of these animals dying in their natural environment would rarely, if ever, be carried out. Since tuberculosis has been found in wild birds in Australia (Seddon, 1953) and in America (Feldman, 1938), marsupials in their natural environment could well have contact with avian type tubercle bacilli, and it is possible that avian type infection does occur.

Summary

A case of tuberculosis in a captive wallaby, caused by the avian type tubercle bacillus, is described. The literature relating to tuberculosis in marsupials is reviewed.

Acknowledgment

I wish to thank Mr. E. H. Tong, Director, Zoological Society of London, Whipsnade Park, and Mr. M. Senior, M.R.C.V.S., who supplied the history of this animal and the material for examination.

REFERENCES

Feldman, W. H. (1938): Avian Tuberculosis Infections, pp. 234-240. London: Baillière, Tindall & Cox.
Griffith, A. S. (1928): *J. Hyg. Camb., 28,* 198-218.
Hamerton, A. (1932): *Proc. zool. Soc. Lond.,* 1932, II, 613-638.
Lucas, N. S. (1925): *J. Path. Bact., 28,* 123-124.
Seddon, H. R. (1953): Diseases of Domestic Animals in Australia. Part 5, Vol. I. p. 154. Commonwealth of Australia, Dept. of Health, Service Publ. (Div. Vet. Hyg.) No. 9.
Stableforth, A. W. (1929): *J. comp. Path., 42,* 163-188.
Stünzi, H. (1950): *Virchows Arch., 318,* 97-103.
Teuscher, E. (1957): *Zbl. VetMed., 4,* 747-793.
Tilden, E. B. & Williamson, W. M. (1957): *J. Amer. vet. med. Ass., 131,* 526-527.

RUPTURE OF THE GASTROCNEMIUS TENDON IN FOWLS

by

R. B. A. CARNAGHAN, M.R.C.V.S., and B. S. HANSON

Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey.

Introduction

Rupture of the gastrocnemius tendons in fowls is a condition which has become increasingly common during the past few years. It was first encouuntered at this Laboratory in 1953 when two cases were seen during the year. Since then the annual number of birds diagnosed with the condition has been 6 in 1954, 15 in 1955, 46 in 1956 and 59 in 1957. Although the figures for the last two years represent somewhat less than one per cent of the total number of adult fowls submitted for examination

each year, nevertheless the condition is of some economic importance as it occurs at, or near, maturity and the morbidity in affected flocks is often about 10 per cent.

The condition is not a new one in this country. Harris (1947) observed it in 1939 when 38 birds were found to be affected out of just over 1,000 White Leghorn pullets examined post-mortem. It has been described in the United States by Bullis and van Roekel (1944) who observed that it was most apt to occur during the spring in out-of-season hatched birds between five and seven months of age. Chute (1950) observed the condition in Canada and reported a 10 per cent incidence in a flock of 600 New Hampshir. X Light Sussex capons. He also cited a further outbreak of the condition which affected about 10 per cent of a flock of Barred Plymouth Rock capons.

None of these workers was able to establish the cause of the condition and Chute (1952), quoted by Jordan (1955), considering the theories on aetiology under the three headings—predation, nutrition and management and genetic factors—did not attach particular importance to any one of these.

Jordan (op. cit.) described the condition which he saw in this country in genetically related fowls on three separate farms and concluded that genetic factors were probably concerned in the aetiology. He found no evidence that injury by predatory animals was a factor but thought that out-of-season hatching might be of some significance as most of the birds which were affected were hatched in the winter months. Nearly all the cases he examined occurred in birds kept in strawyards or on deep litter and he suggested that these two systems of management might favour rupture of the gastrocnemius tendon in birds with an inherited tendon weakness.

This paper describes a small breeding experiment which was undertaken to determine whether genetic factors are concerned in the aetiology and to ascertain to what extent environmental conditions are contributory factors.

Experimental

Three Light Sussex pullets were received alive showing symptoms of lameness. One of these birds had bi-lateral rupture of the gastrosnemius tendons while in the other two the condition was unilateral. In each case the ruptured end of the tendon could be palpated just above the tibio-metatarsal joint. Shortly afterwards a R.I.R. cockerel was obtained from a different source with bi-lateral rupture of the tendons. In each bird the rupture was of recent origin and the birds were placed in coops for about a month so that some degree of repair of the tendon could take place. They were then housed in a fold unit, on grass and fed a proprietary brand of breeder's mash. Eggs were incubated weekly in an electric table incubator and the resulting chicks reared under infra-red lamps for three weeks. One group of 15 chicks was brooded in a Cope's electric brooder to determine whether brooding methods had any influence on the incidence of the condition. At the end of this period they were kept for a further time indoors, depending upon prevailing weather conditions, before being divided into two groups, one of which was reared in fold units on grass while the others were reared intensively in wooden huts on sawdust litter. There were no perches in either type of accommodation and when the birds commenced laying, the nest boxes were placed on the floor to obviate any strain on the tendons. Both groups received the same proprietary brand of food. Chicks were hatched each month from October until the following February.

XUM

During the course of the experiment 12 cockerels between the age of six and a half and eleven months were caponized by chemical means in order to control fighting which had broken out in the group. In view of Chute's report (op. cit.) of the condition in groups of capons it was thought that this would also help to determine whether chemical caponization increased the incidence of ruptured tendons in susceptible birds.

Due to limited accommodation it was not possible to maintain a control group but ruptured tendons have never occurred in many hundreds of fowls previously reared in the same manner and fed on a diet similar to that used for the experimental birds.

Results

Two hundred and ten eggs were incubated; 21 of these were found to be infertile on candling after six days and from the remainder a total of 116 chicks was hatched. Due to an outbreak of caecal coccidiosis among those housed intensively and a number of deaths in the birds on range from impactions of the crop and gizzards only 70 of

TABLE 1

Age in Months	Number Affected	Sex
4½	1	♀
5	3	2♀, 1♂
5½	2	2♀
6	2	1♀, 1♂
6½	1	♂
7	2	2♂
7½	2	2♂
8	-	-
8½	-	-
9	1	♂
9½	1	♂

these birds survived to three months of age. Fifteen of these 70 survivors developed ruptured tendons. Six of these were pullets and nine cockerels. Six cases occurred in the intensively kept group while the other nine were in the birds on grass. The youngest bird affected was aged four and a half months while the oldest was nine and a half months. Table I shows the ages at which rupture of the tendons occurred.

This age incidence is in agreement with that observed in field outbreaks by other workers but it also suggests that pullets become affected at an earlier age than cockerels.

Table II shows the month during which hatching occurred and the number of birds which subsequently developed ruptured tendons.

TABLE 2

Month	Total Chicks Hatched	Number Subsequently Affected
October	22	4
November	30	5
December	24	4
January	21	0
February	19	2

* Severe mortality occurred in the January group. Only six birds survived to three months of age.

The months during which rupture of the tendons occurred were as follows:

	February	March	April	May	June	July	August	September
Number of cases	1	0	1	6	2	4	0	1

In 10 of the birds the tendons ruptured in both legs, in two the right leg only was affected while in the other three the left leg was affected. In most of the bi-lateral cases the ruptures occurred simultaneously but in two instances birds with a rupture of the tendon in one leg were placed in coops where movement was restricted and in each bird the other leg became affected, in one after 24 hours, in the other after an interval of 11 days.

One case of ruptured tendons occurred in a cockerel after it had been in a coop for 14 days. The bird had been removed from its pen as it was being severely bullied by other cockerels.

Two of the cases developed in the birds which had been brooded in electric brooders. None of the cockerels which were chemically caponized became affected.

Post-mortem examination of affected birds failed to reveal evidence of any other disease. Thirteen of them were in good bodily condition with moderate deposits of fats in the abdomen and mesentery while the other two were rather poor. Four of the six pullets were in lay while in the others the ovary was becoming active.

In the majority of cases the rupture had occurred at a point just above the insertion of the component of the gastrocnemius tendon into the tuberosities on the proximal extremity of the metatarsus. The elasticity of the tendon had caused it to recoil above the angle of the joint in which position the ruptured end of the tendon could be palpated. Tendon rupture was invariably accompanied by extensive subcutaneous haemorrhage above the joint.

XUM

Discussion

Investigations by other authors into the aetiology of rupture of the gastrocnemius tendon in fowls, based on field observations, have suggested an inherited predisposition to the condition. Bullis, quoted by Chute (1950) noted that it occurred more often in certain breeds and strains than in others. In 1950 Chute considered, on the information then available, that nutritional or genetic causes were indicated, although in 1952, quoted by Jordan (1955), he did not then attach particular importance to either of these factors. Jordan's investigations in this country led him to believe that genetic factors were concerned in the aetiology, although he believed that out-of-season hatching, environmental conditions and early maturity were contributory causes.

The results of the present experiment suggest that the condition is basically due to inherited factors. In the published descriptions of field outbreaks an incidence of approximately 10 per cent. in affected flocks has been almost constant. Bullis and van Roekel reported 10 per cent. in their case; Hancock and Ballantyne, quoted by Chute (1952), found 10 per cent. of 1,000 birds affected. Chute himself found 10 per cent. of 600 Hampshire Red X Light Sussex birds affected with the condition and in Jordan's case 9.2 per cent. became affected. The only exception to this would appear to be the outbreak described by Harris (1948) where the incidence was 3.8 per cent.

In our experiment the incidence of affected birds was 21 per cent. which is double that reported in field outbreaks. This figure is consistent with an inherited cause as the parents on both sides were affected, whereas in field cases it would be reasonable to assume that the factor for tendon weakness, due to its comparative rarity, would be possessed by only one of the parents.

The result of most of the previous investigations suggest that other factors besides genetic ones play some part in the aetiology of the condition. Out-of-season hatching has been suggested by Bullis and van Roekel and by Jordan. The first mentioned workers found in one flock that birds hatched in October and January developed ruptured tendons while those hatched in March, May and June did not. Similarly Jordan (op. cit.) found that in three outbreaks which he investigated most of the birds which were affected were hatched during the winter. However, in 1957 the majority of cases which he investigated occurred in birds hatched in March and April (Jordan, 1958). In Chute's report the chicks were hatched during April. It is difficult to appreciate what effect winter hatching could have on chicks particularly under the present day intensive method of housing and management. It is suggested, as a more reasonable explanation, that the condition shows a distinct seasonal incidence and that hatching times are coincidental to this. In our experiment 80 per cent. of the cases of ruptured tendons occurred during May, June and July. During 1956 61 per cent. of field cases diagnosed at this Laboratory occurred during these three months, the remaining 39 per cent. being spread over a period of seven months. In 1957, 66 per cent. of the total annual number of cases were diagnosed during the same three months. It is considered that previous reports also support this view. Bullis and van Roekel found that the condition was most likely to occur in the spring and Chute found a 10 per cent. incidence of the condition at the time a flock was inspected in mid-August. In Jordan's report it is possible to calculate the time of rupture of the tendons to May, June and July from the information given of the hatching dates and age incidence.

The experimental results suggest that pullets tend to become affected at an earlier age than cockerels and this may be associated with the slower rate of development of males particularly with regard to the age at which they reach sexual maturity. Both field and experimental observations show that rupture of the tendons occurs at an age when sexual development is at a maximum and it is considered that the seasonal incidence observed is the period during which this occurs naturally in the fowl. It is suggested, therefore, that the influence of sex hormones may be a secondary factor in the aetiology of the condition. The fact that none of the chemically caponized cockerels developed ruptured tendons is considered to support this opinion. By the time the effect of hexoestrol had waned the birds had reached an age beyond that during which the condition occurs. In Chute's case however, ruptured tendons occurred in birds between two and a half and four months after caponization by which time, if the caponization was by chemical methods and this is not stated, the effect would have worn off and normal testicular activity would have recommenced.

On the other hand it should be stated that we have twice encountered the condition in the field in chicks as young as six weeks of age for which no logical explanation could be found.

The previous evidence on whether conditions of management are of any significance in the aetiology is conflicting. Jordan (1955) found that nearly all his cases occurred in birds kept in strawyards and on deep litter. On the other hand, Chute reported the condition in birds on range having access to grass. The results of our experiment, where there was little difference in the incidence between the two groups, suggests that the type of management is not a significant feature in the case of ruptured tendons.

Although Bullis and van Roekel and Jordan consider that strain on the tendon caused by jumping on to perches and into nest boxes and scratching in litter might be associated with the condition, the evidence presented here does not confirm this view. In both experimental groups of birds steps were taken to ensure the minimal strain on tendons. Further, in one experimental bird rupture of the tendons occurred after it had been isolated in a coop for 14 days under conditions where there was little opportunity for exertion of the tendons to occur. In two other instances, birds were placed in coops having ruptured the tendon in one leg and rupture of the other tendon occurred in one case one day later, in the other after 11 days while the birds were recumbent from the effect of the original lesions.

The writers appreciate that these experimental results are open to criticism in that no control birds were kept. This was due to limited accommodation being available and it was considered preferable to rear a comparatively large number of experimental birds rather than to rear half this number using the remaining available space for controls. However, many hundreds of other fowls have been reared at this Laboratory throughout the year by the methods described, in the same accommodation and fed on a similar diet without ruptured tendons having occurred. Despite the omission of a control group it is thought that the results of this experiment indicate that rupture of the gastrocnemius tendon in fowls is primarily due to inherited factors. It is suggested that the influence of sex hormones may be a secondary factor concerned in the cause of this condition.

XUM

Summary

A breeding experiment was carried out using as parent stock fowls which were affected with rupture of the gastrocnemius tendon. Fifteen (21 per cent.) of the 70 offspring which survived to three months of age subsequently developed tendon rupture. It is suggested that the condition is due to inherited factors and that the time of rupture is influenced by the activity of the gonads.

Acknowledgments

To Mrs. S. Langley and Miss H. Chilcott for technical assistance.

REFERENCES

Bullis, K. L. and van Roekel, H. (1944): *Cornell Vet., 34,* 4.
Chute, H. L. (1950): *Can. J. comp. Med., 14,* 218.
Harris, S. T. (1947): *Vet. J., 103,* 356.
Jordan, F. T. W. (1956): *Vet. Rec., 67,* 548.
Jordan, F. T. W. (1958): Personal communication.

REVIEWS

BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY, by R. S. Breed, E. G. D. Murray and N. R. Smith. Seventh edition. London: Baillière, Tindall and Cox. Price 120s.

BERGEY'S Manual is unique; no other volume attempts to catalogue and describe all currently recognised bacterial species, and Bergey thus assumes the same significance for the bacteriologist as " BEPS " for the zoologist, or Wisden for the cricketer.

The present edition is smaller and costlier than before. The ungainly four and a half pound volume published in 1948 contained over one hundred appendices of inadequately described strains and *species incertae sedis;* these, together with the host and habitat lists are being relegated to a second volume to be resoundingly called the INDEX BERGEYANA. Another not unexpected casualty has been the classification of viruses, with its romantic binomials to which so many people took exception (louping ill, for example, had become *Erro scoticus* meaning the Scots vagrant, and this was felt by some to be going a little far).

Veterinary bacteria on the whole are dealt with accurately. The organism causing nephritis in foals is now placed in the genus *Actinobacillus,* with the specific epithet *equuli,* but bacteriologists are accustomed to the frequent name changes of this species. *Corynebacterium renale* is still described mainly from the account of Jones and Little, published over thirty years ago, and consequently two important features (urease production and the effect on milk agar) have been left out. The highly characteristic C.A.M.P. test for *Streptococcus agalactiae* is also omitted. It is understandable, however, that in a work of this size textual errors must occur, and indeed they are so few as to scarcely blemish this otherwise admirable edition of a standard reference work.

ANIMAL BEHAVIOUR, Vol. VI 1 & 2, 1958. Formally *British Journal of Animal Behaviour.* London: Bailliere Tindall & Cox.

DURING recent years the study of animal behaviour and response to environment has attracted the attention of an increasing number of workers in the scientific field. This interest stems from and is correlated with the efforts that have been made to seek explanation for many of the psychological disorders of man which, since time immemorial, had been attributed to the displeasure or fancy or whim of a divine providence. These disorders were accepted as things which were in part inevitable, just as in the realms of organic disease, and before Pasteur shed the illuminating rays

which were the foundation of the germ theory, a certain percentage of mortality amongst man and animals was accepted as normal and allotted its own line in the economic balance sheet. The study of animal behaviour is worth-while for its own sake but there is a greater field in which it may be of inestimable value, i.e. in the elucidation of behavioural and mental troubles of man. Fundamental knowledge acquired in the study of animals may open the door and disclose and explain some of the as yet inexplicable reactions of the human mind.

The Association for the Study of Animal Behaviour was founded in March 1936. It started in a modest way but the discussions were unique and extremely interesting to those of scientific bent, although some of the material which was proclaimed by men of science had been common knowledge to lay stock-keepers for a very long time. The striking feature to one who had been familiar with the behaviour of horses all his life and by chance entered one of the meetings whilst a very distinguished scientist was dissertating on certain actions and habits of these animals, was the new meaning which commonplace habits acquired when they were analysed scientifically.

It was not long before the Association required an official publication by which to record the members' observations and the *Bulletin of Animal Behaviour* was born in 1938. In spite of the general upset occasioned by World War II nine issues had been published by 1951.

The study of animal behaviour was not confined to this country and was also pursued actively in America. It was realised that a regular journal was required and at first the Association gave their support to the international journal *Behaviour*. The Association desired its own publication and the *British Journal of Animal Behaviour* was launched in 1953. This journal attracted contributions from many other parts of the world and particularly from America and after a time it was realised that the title British did not represent fully the wide appeal of the periodical. American and British workers got together and resolved to collaborate in the introduction of a new journal and under a title which was not insular. *Animal Behaviour* is the outcome; it is published according to the high standards demanded by the *British Journal of Animal Behaviour* and in fact it has inherited the serial volume number of the latter journal. The first issue is Vol. VI, 1 & 2, Jan.-April 1958. The Editorial staff include British and American workers. This, the first issue under the new title, is produced handsomely and is published in London. The articles in the text range over a wide field from the octopus to the domestic hen and come from authoritative sources.

Successful veterinary surgeons have to be adept in two branches of psychology, that of the animals and, no less important, that of the owners. I am sure that many colleagues and especially those of a philosophical turn of mind will fine much interest in this journal which is devoted to animal behaviour.

TALKING ABOUT DOGS, by Olwen Gwynne-Jones. London: Faber & Faber. Price 12/6.

DOGS cannot talk and give opinions on their own management or that of their two legged masters. This may be a good thing and save a lot of doggy breath for their masters talk so much about dogs that the beasts would not be able to insert a word even edgeways. The experts who breed and show dogs excel in this form of "natter" and most of the noise at a dog show does not come from the dogs. The professionals dissertate with verve and cunning using a curious jargon which adds a spice of mystery to the topic and serves to keep the untutored tyros in a state of wonder and requisite humility.

Much of the conversation is just "Bunk" delivered with a showmanship which would make that great psychologist the "Witch Doctor" bow his head in deep respect. In spite of these facetious remarks I acknowledge that amongst dog owners and breeders there is a great fund of valuable and most useful knowledge which is founded on sound personal observation or has been acquired in the traditional way from some preceptor of long experience. It is evident that Miss Gwynne-Jones is an acute

XUM

observer and she is a gifted scribe and preceptor. In this book of 20 short chapters she has covered every facet of the dog breeders world including housing, feeding, breeding, whelping, diet, the management of stud dogs, showing and judging. On all these things she writes with an authority based on personal experience. The script is lucid and there is no waste of words. This book should be a most useful one to any newcomer into the dog world and there is no doubt that experienced dog breeders and even veterinary surgeons will find interest and profit within its pages.

DAIRY CATTLE BREEDING IN THE TROPICS, by P. Mahadevan, Ph.D., Tech.Comm. No. 11, Commonwealth Bureau of Animal Breeding & Genetics, Edinburgh. Pages 88, illustrations 14, price 15/-.

THE author of this book, which takes the form of a survey of literature on Dairy Cattle Breeding in the Tropics, is a man with considerable experience of his subject. As stated in the preface many of the observations made are inconclusive and interpretation can be no more than plausible, but that is not the fault of the author who has succeeded in assembling and summarizing the available data in an exemplary manner.

The various problems of milk production, breeding, types of cattle, environment and genetics are discussed fully, and the book helps one to realize the many problems and the vast amount of research which is still necessary if the breeding of dairy cattle in the tropics is to be improved.

NEWS

Mr. B. V. Jones, M.R.C.V.S., has been appointed Head of the Veterinary Research Department of Allen & Hanburys Ltd.

NOTICES

BURROUGHS WELLCOME & CO. announce the introduction of 'Trivexin' brand Braxy, Blackleg, Pulpy Kidney Vaccine for the vaccination of sheep in areas where all three diseases are prevalent. 'Trivexin' is available in bottles of 250 c.c. (50 doses).

They also announce the introduction of 'Wellcome' brand Improved Lamb Dysentery Vaccine for the protection of new-born lambs, via the colostrum, against pulpy kidney disease and lamb dysentery.

The use of this new vaccine can be so adapted that the adult sheep themselves can be protected against pulpy kidney disease and "struck". The vaccine is given in a dose of 2 c.c. and is available in bottles of 100 c.c.

GLAXO LABORATORIES LTD. announce the introduction of Biotexin Cerate as from Monday, 18th August. Biotexin Cerate contains novobiocin in an oily base with 3% aluminium stearate, each tube delivering 0.25 grams of novobiocin which is present as the monosodium salt. The product is available in a pack of 12 tubes.

Novobiocin has an antibacterial activity which can be compared with penicillin. Its principal value in mastitis is in the control of staphylococcal injections which are not susceptible to other antibiotics.

They also announce a price reduction as from Monday, 18th August for Predsolan Tablets (available in bottles of 25 tablets), and price reductions from 18th August on their systemic, topical and ophthalmic corticoid preparations. Altogether 26 separate packs are affected by the reductions, including the ointments, tablets, lotions, drops and also the new veterinary pack of Predsolan tablets.